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APRIL, 1918

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ARNOLD THEILER,
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Acute Liver-Atrophy and Parenchymatous Hepatitis in Horses.

BY

SIR ARNOLD THEILER, K.C.M.G.,
Director of Veterinary Research.

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By SIR ARNOLD THEILER, K.C.M.G., Director of Veterinary Research.

THE object of this paper is to describe a disease amongst horses noticed in South Africa during the last three to four years, commonly called acute Staggers or Malziekte respectively in English and Dutch, and which we hold to be identical with the acute Liver-atrophy of man. The disease described under this name in the veterinary literature does not correspond with that under discussion, as will be shown in the course of this paper. Under the name of parenchymatous Hepatitis a disease is described that was produced artificially by feeding horses on *Senecio latifolius* (D.C.), or ragwort, which plant was, and still is, commonly identified as connected with the production of the Dunziekte of horses, otherwise also known as Grass-staggers or Ragwort poisoning. It is not the intention to deal with Dunziekte in this paper, as a detailed account will be given in a future report; suffice at present to state that Dunziekte is a cirrhosis, whilst Ragwort poisoning is a parenchymatous Hepatitis. The placing of the acute Liver-atrophy and parenchymatous Hepatitis under the one heading is done with the object of showing that, in our opinion, there is a certain relationship between the two, in particular from the pathological anatomical point of view, which will help to throw some light on the ætiology of acute Liver-atrophy. The subject will be treated under the headings of (1) acute Liver-atrophy and (2) *Senecio* poisoning, where the anamnesis, pathological anatomy and symptomatology will be treated in some detail, (3) the ætiology of Liver-atrophy will be discussed with reference to experimental research on the one hand and the analogy to *Senecio* poisoning on the other.

I. ACUTE LIVER-ATROPHY.

1. *The acute Liver-atrophy of Human Pathology.*—Human pathology describes an acute (genuine) Liver-atrophy, which is characterized microscopically by an extensive fatty degeneration or a granular necrosis of the liver cells. The course of the disease is stated to last from a few days to one or two weeks. An acute yellow and a red atrophy are distinguished. In the acute yellow or genuine atrophy the liver is reduced in size and in weight, sometimes by more than one-half. The organ is flat, the borders are sharp, and the consistence flabby. The consistence may, however, also be tough (Kaufmann). The capsule is shrivelled. Acute and subacute cases are recognized; the former are characterized by the intensely yellow or ochre yellow colour, whilst the subacute cases show yellow and red foci, often not sharply defined. The red foci in cases of longer duration are found increased in size and crowd the yellow ones out, so that finally only a few yellow specks on a reddish background are seen (red atrophy). The yellow streaks are sunken. A lobulation in the red portion can no longer be recognized. The microscopical examination reveals the presence of an extensive fatty degeneration of the liver cells and of necrosis in the central portion of the lobule, indicated by a granular detritus. In acute cases the periphery of the lobules shows more or less intact or fatty degenerated hepatic cells; in advanced cases, however, the whole lobule is involved, so

that only the capillaries and between them the nuclear detritus, containing fat droplets and pigment, can be seen. These capillaries are distended with blood, but extravasation into the lobules also takes place. Thus the picture of the red atrophy is produced. At the same time in the red portions a small round cellular infiltration of the interlobular connective tissue and a considerable proliferation of the bile-ducts takes place, these in forms of peculiar long multinuclear epithelial cells. We can summarize the lesions found: *Icterus, fatty degeneration and necrosis of the liver cells, stasis and extravasations; Emigration of round cells and regeneration of bile-ducts.*

Symptoms.—Two stages are distinguished. The first one presents the lighter prodromal, of which icterus is one, the second the characteristic grave symptoms. Of importance is the latter. It is characterized by grave, nervous symptoms: heavy headache in conjunction with general restlessness and insomnia. The patients are soon delirious, the delirium rapidly increasing and going over into complete mania. The patients are very restless, and their mania can sometimes only be mastered with greatest difficulty. Muscular spasms and sometimes, but by no means frequently, epileptiform attacks are noted after one to two days, rarely later. The excitation gives way to a soporous state going over into coma. Rarely the excitation is absent and the disease begins with the grave nervous symptoms of sopor. The symptoms may be summarized: *Icterus, excitation, sopor, and coma.*

2. *The acute Liver-atrophy in Veterinary Pathology.*—In the textbook of Kitt the acute yellow and red liver atrophy of domesticated animals is not described. In an appendix to the description of icterus, he gives the description of the human disease. Friedberger and Fröhner treat it in an appendix to the parenchymatous Hepatitis and consider it to be identical in most cases with that of Lupinosis. The pathological lesions are stated to be about the same as those found in sheep; however, a more severe inflammation of the intestinal track is stated to be present in this disease. An extract of the more important pathological lesions of Lupinosis in sheep is given: (1) Acute parenchymatous Hepatitis: a granular albuminoid cloudiness of the liver cells is present, the liver being either enlarged or normal. The initial stage is succeeded by fatty degeneration of the liver, this organ being flabby, soft, and friable. The next stage is that of the acute yellow atrophy, in which the softened liquid liver cells are resorbed, the liver decreasing in size. The evolution period for this stage is considered to last about fourteen days. (A chronic Lupinosis consisting in an interstitial Hepatitis is also mentioned.) (2) Icterus. The liver is either light or lemon yellow, red, or ochre yellow. Subcutis, peritoneum, omentum, and mesentery may also be yellow. A detailed description of the microscopical changes is not given. The clinical symptoms in lupinosis of the horse are given as follows: loss of appetite, dull sensorium, symptoms of a grave pressure of the brain can follow, the animals from time to time placing the head on the manger, grinding the teeth, stepping back from the manger, hanging of the head, and uncertain swaying gait. A low fever is present, which occasionally goes over to high fever; icterus is noted; defecation is rare, micturition frequent in small quantities. These symptoms last about eight days, and recovery is complete after sixteen to twenty-one days. *Fatal cases have not been observed.* A comparison of both the anatomical and clinical symptoms given as those

of Lupinosis of the horse with those of acute Liver-atrophy of men shows that they cannot be considered to be identical. Hutyra and Marek deal in a special chapter on acute yellow Liver-atrophy. The pathological description given corresponds with that found in the textbook of human pathology and is quoted above. The clinical symptoms given are as follows: The disease begins with rapidly increasing somnolence and depression, and occasionally with febrile symptoms, succeeded soon by icteric discolouration of the mucous membranes. Loss of appetite and occasionally symptoms of colic are present, and sometimes also diarrhoea, whereupon soon the icterus, stupor, and lassitude reach a high degree. Symptoms of excitations are occasionally present. In consequence of the simultaneous Nephritis the quantity of urine is reduced, the urine becomes reddish-brown in colour and contains albumen and bile pigment. As a rule in six to eight days the disease ends in death. The decrease in the quantity of urine and the appearance of albuminuria have to be considered as unfavourable symptoms. The disease described by Hutyra and Marek is undoubtedly one of the liver. There is no evidence to show that the description of the pathological histology given by them refers to that of the horse. Probably this is not the case. The authorities quoted date back to Adma (1857), Callot (1880), Delage (1838), Franze (1862), Zundel (1854); only two are of more recent times, i.e. Beauvais (1894) and Stoll (1901). This literature is, however, not accessible to me. In the light of my own experience, I am not inclined to identify the symptomatology of Hutyra and Marek with their pathological anatomical description and do not consider the disease described by them as acute Liver-atrophy, notwithstanding the presence of some of the symptoms, which might be ascribed to that disease.

3. *The acute Liver-atrophy as observed in South African Horses.*

History and Anamnesis.—In November, 1914, the disease "Staggers" was diagnosed by us for the first time and identified with acute Liver-atrophy. Since these cases were noted in horses that had passed through a horse-sickness experiment, we concluded provisionally that the disease was connected with the method of immunization. This opinion had to be abandoned when the disease was noted in a troop of horses that were not immunized, and we concluded then that its appearance in inoculated horses was a mere coincidence. Accordingly we continued the immunization of horses in the practice, but finally had to withdraw the immunization, since there was no doubt that Staggers appeared amongst the inoculated horses in an alarming degree, that was out of proportion to the cases in non-inoculated horses. Our observations can be dealt with under three different headings: Acute Liver-atrophy observed in (1) horses at Onderstepoort, (2) in horses belonging to the Defence Force, and (3) in horses of farmers in the districts.

(1) *Staggers observed at Onderstepoort.*—In the tabulated form attached a résumé of the history of horses is given that contracted Liver-atrophy during the period under consideration. The first case definitely recognized dates back to November, 1914. Since 1915 the cases have been of fairly frequent occurrence. The last case included in the tabulated form is dated the 17th June, 1917. It is a remarkable observation that Staggers was noted amongst horses that previously had been immunized as well as those not immunized, and this latter fact must be emphasized, as it is of importance for the

explanation of the cause of the disease. Dealing with the non-injected horses first, we note that they arrived at Onderstepoort in two batches on the 27th November, 1915, and 4th December, 1915. On the 24th January, 1916, they were utilized in a dipping experiment for the purpose of ascertaining whether dipping would act as a preventive for Horse-sickness. They were taken ill on the 37th, 68th, 78th, and 89th day after the commencement of the experiment. One of these horses recovered (9880) and was immunized subsequently against Horse-sickness. The rest of the horses had all been immunized against Horse-sickness with serum and virus, with the exception of 8762, which received only virus, and 11046, that received only serum.

The number of cases observed at Onderstepoort during this period amounted to 31, of which 25 died and 6 recovered. One horse (9571) had a second attack, which clinically corresponded to Staggers, but the results of the autopsy were conflicting. The mortality calculated on the number of horses subjected to similar treatment during the corresponding time is as follows: Four cases, with one recovery, amongst 160 horses that were dipped, the mortality thus being 1.9 per cent.; 27 cases (of which 22 died) is the total number amongst 1148 horses that were injected during the same period, the mortality amongst these is thus approximately 2 per cent. The horses suffering from Staggers were in all cases geldings, except one which was a stallion. With the exception of the stallion, which was five years old, one gelding of six years, three of seven years, and three of eight years, they were mostly aged horses, and included all colours: chestnuts, bays, browns, greys, and creams. The majority of these horses were obtained from the Defence Force; they had either been in Capetown or had been running previously on farms near Immigrant in the Free State. It is possible that some or all of them had been through the German West campaign. The immunized horses that developed the disease did so a certain time after the injection of the virus causing the Horse-sickness reaction. A Horse-sickness reaction was not noticed in one instance (9581), and, of course, did not develop in the horse only injected with serum (11046). The intervals between inoculation and outbreak of disease were as follows: 27 days is the shortest and 165 days the longest instance; they mostly occurred between the 62nd and 78th day, or, more accurately, on the 62nd day (two cases), 64th, 66th (two cases), 68th (two cases), 69th, 71st (three cases), 73rd, 74th, 76th, 78th, 79th, and on the 80th, 82nd, 84th, 85th, 87th, 89th, 91st, 94th, and 97th day. It is a remarkable observation and one that must be looked upon as of not mere coincidence, that horses, belonging to the same batch of treatment, viz., injected at the same date, broke out within a few days of each other. On the 7th September, 1915, 97 horses were injected, and of these two succumbed (9581, 9482), one on the 71st and the other on the 69th day. Of the 85 horses injected on the 18th December, 1915, two developed the disease on the 62nd (9994) and 66th days (9962) respectively, two on the 71st (10006) and 78th (9989) days, and two on the 91st (9949) and 94th (9938) days respectively. Of a batch of 96 injected on the 8th February, 1916, two developed the disease within two days of each other, viz., on the 74th (10159) and 76th (10094) day. This observation naturally led to the conclusion that the inoculation, or at least Horse-sickness, is somehow connected with Staggers. Comparing, however, the percentage of the outbreaks amongst not immunized horses with that amongst immunized horses, there seems to be no foundation for such an assumption. The fact that in our

stables Staggers was found more amongst immunized horses is explained when it is pointed out that, with the exception of the dipped ones, our horses were used for immunization experiments soon after arrival. The horses subjected to immunization with serum and virus were treated in a similar manner, except 9632, which received an initial virus, that called Onderstepoort strain of the first generation, whilst the bulk received as initial virus that of the Tzaneen strain of the 12th, 13th, or 14th generation, and as a second virus that of the Ordinary strain (Pretoria) of the 175th generation (15 horses) or 176th generation (10 horses). One horse 10641 was tested on its immunity by three viruses, Tzaneen 107th generation, Onderstepoort 3rd generation, and Bulawayo 14th generation. Of the immunized horses 12 were later hyperimmunized. One of the dipped horses that recovered was later also immunized without ill-effects. In four horses *Nuttallia equi* was found during a biliary fever reaction, and one was complicated with *Spirochaetes*. Four horses of the hyperimmunized lot proved to have haemolytic qualities in their serum and were, therefore, rejected for serum use. The serum which was utilized for the immunization was that known as (O.) Serum 1711 or 1712, 1712A, 1713, 1714, 1715, and 1718. The serums 1711 to 1713 were made of horses that were hyperimmunized three times at 10,000 c.c. per time, and the different batches were mixed at the conclusion of the last collection; serums 1715 and 1718 were obtained from three different batches of horses, one hyperimmunized once, one that was twice and one three times hyperimmunized. The number of horses in each batch numbered about 30-40.

Subsequent to June, 1917, the immunization of horses was somewhat changed, and for virus O. a mixture of Onderstepoort and Tzaneen was substituted as a second virus, the horses, however, still receiving Tzaneen virus as a first injection. Of 31 horses treated in this way two succumbed to Staggers: one on the 87th and one on the 81st day after immunization. Three horses only received Tzaneen virus as a first virus and no second virus. Of 35 horses treated in this way three developed Staggers, one contracting it 81 days, one 122 days, and one 97 days after immunization. A further noteworthy observation is the one in horse 11485 which received virus "O." exclusively of the 182nd generation and died of Staggers 84 days later. All these horses as a result of virus injection had passed through a Horse-sickness reaction. The most remarkable case, however, is the one of 11543, a gelding that was infused with 5000 c.c. blood of horse 11278 that 61 days previously had been immunized against Horse-sickness. It was susceptible to horse-sickness, and after the infusion showed no reaction whatever. It developed Staggers 34 days after infusion, and died of the disease. This case thus represents a Staggers case in a non-immune horse. It is further remarkable that Staggers was noted after June, 1917, only in horses bought from horse-dealers, whilst during the same period 180 horses obtained from the Defence Force were immunized, amongst which no Staggers was noted.

Summary and Conclusion.—It will be noted that there is no common factor applying to the horses in which Staggers was noted. It was observed in four horses out of 160 that were never submitted to any inoculation, in two horses that received only an injection of virus (one virus Tzaneen and one O. virus), and in one that only received serum, whilst the greater number had received both serum and virus, viz., Tzaneen and O. virus. It was also noted after immunization with Tzaneen virus and serum, after Onderstepoort and O. virus and

after Tzaneen and Onderstepoort and Bulawayo viruses. It is further interesting to note that the morbidity due to Staggers since the diagnosis of the first case on the 21st November, 1914, amounted to 27 cases, including five recoveries (the details of which are all attached in the appendix) out of a total number of 1148 horses immunized until end of June, 1917, viz., 2.2 per cent., which figure differs only slightly with the one observed in horses not treated. (Details of the cases observed after June are not given.) It is remarkable that the disease was only noted since November, 1914, whilst the immunization experiments have been going on for some years before, in which quite a number of horses recovered and were subsequently kept under observation. From June, 1910, to June, 1914, a total number of 840 horses that had recovered from Horse-sickness, were kept over a period of three months under observation, yet the occurrence of Staggers was not diagnosed, and if it had occurred it could hardly have been missed. The only difference between the immunization of the horses previous to November, 1914, was the use subsequently of (O.) virus of a very high generation in the Staggers horses, but this difference, however, cannot explain much, since the disease was also noted in horses not injected at all. The only conclusion which can be drawn from the Onderstepoort observation is, that the acute Liver-atrophy is a new disease of horses that occurred only fairly recently and that it is not due to the immunization of horses, although it is sometimes noted after this process.

In Onderstepoort, therefore, the disease Staggers was noted under the following conditions:—

- (1) In horses not immunized.
- (2) In horses after injection of Tzaneen virus (T. virus).
- (3) In horses after injection of Ordinary virus (O. virus).
- (4) In a horse after injection of O. serum.
- (5) After injection of Tzaneen virus and O. serum.
- (6) After injection of Tzaneen and O. virus and O. serum.
- (7) After injection of Tzaneen, Onderstepoort, and Bulawayo virus.
- (8) After injection of Onderstepoort and O. virus and O. serum.
- (9) After an infusion with blood of a horse immune to T. and O. virus.

(2) *Acute Liver-atrophy in Horses belonging to the Defence Force.*—From the 1st March, 1916, to the 6th January, 1917, a total number of 1411 horses were inoculated against Horse-sickness. The method employed was in all cases identical, although the serum and virus varied in different batches. The mortality due to Staggers was given amongst these horses as 51. Of this number one died in Durban. The horses were kept under observation four to six weeks after discharge from immunization, viz., for a period of two to two and a half months after injection. The horses were then shipped to German East Africa, and no further records were obtainable. Since in the experience of Onderstepoort outbreaks of Staggers may occur up to three months after immunization and later, these figures are not quite accurate, and the mortality is most likely somewhat higher. On the other hand the diagnosis Staggers did not in all cases refer to the disease acute Liver-atrophy, but included also acute cases of Dunzietke (Cirrhosis). Of 21 cases submitted for histological examination with the diagnosis "Staggers," 12 proved to be acute Liver-atrophy and 8 Dunzietke (Cirrhosis), and one case a necrosis of the central liver

cells, accordingly the figure of 50 would have to be reduced by about half, and the corrected mortality would then amount to almost double that observed in Onderstepoort. Notwithstanding this inaccuracy of the figure some evidence is obtained of the economical importance of this disease has in inoculated horses. Its presence amongst non-inoculated horses was also noted, but here no statistics are available to draw comparisons by percentages. Unfortunately at a time when a severe outbreak occurred amongst a troop of horses that had been grazing previously at Immigrant, the same farm from where our horses came that contracted Staggers without immunization, no material was submitted for histological examination. Since then, however, practically all the livers of horses that died of Staggers—both in Pretoria and Durban—inoculated or not inoculated, were examined histologically, and amongst these, three cases were found to have died of acute Liver-atrophy. A histological description of the post-mortem of these cases is given in the appendix. The previous history of these horses is not known in two instances, whilst the third one, observed in Durban, was in a horse that a year previously had been bought in Vryheid, Natal, and had been kept for a year at the Remount Depot, Durban.

Conclusions.—Acute Liver-atrophy was noted with certainty in inoculated and non-inoculated Defence Force horses. The mortality amongst the inoculated horses (4·5 per cent.) was greater than that observed in Onderstepoort.

(3) *Acute Liver-atrophy in Horses belonging to the Farmers in the Districts.*—The observations made will be dealt with under the headings of the localities.

1. *Johannesburg.*—On the 6th December, 1916, 22 horses were inoculated. Of these two died of Staggers; both were geldings, one a five-year-old. The animals died 59 and 78 days respectively after inoculation. The diagnosis was made from the description of the cases. The mortality amongst these horses amounts to 8 per cent. (Serum 1715, virus 10451 Tz. 14th gen. and 10437 O. 183rd gen.)

2A. *Pretoria, Brits, and De Wildt.*—On the 17th and 23rd October, 1916, 63 horses were inoculated and of these 12 died. Of this number one was a mare; the ages ranged from four years upwards. Six of these horses died within the 55th and 58th day after inoculation, and the remaining ones respectively on 63rd, 68th, 71st, 80th, 113th, and 126th days. After discharge from the inoculation all these horses were reported to have been running day and night, and most were in daily use. The main mortality occurred between the 17th and 29th December, subsequent to a heavy rain on the 16th December, 1916, and the farmers were inclined to connect the rain with the disease, all the horses having been exposed to it. The diagnosis was made from the clinical description of the cases and the report of a veterinary research officer, who made an inspection *in loco*. Some of the horses were reported to have shown haemoglobinuria. The mortality amounts to 19 per cent. (Serum 1715 and 1717, virus 10215 Tz. 13th gen. and 10036 O. 176 gen.—10219 Tz. 13th gen. and 10437 O. 183rd gen.)

2B. *Police Horses, Pretoria.*—On the 20th November, 1916, 45 horses were inoculated. One horse died of Liver-atrophy on the 18th January, 1917, viz., 59 days after inoculation. It was a gelding. The microscopical examination of the liver in this case was made and supported the diagnosis. These horses had constantly been kept in the stable and were thus under favourable conditions. The mortality

thus amounts to 2 per cent. (Serum 1715, virus 10215 Tz. 13th gen. and 10036 O. 176th gen.)

2c. *Laboratory.—Farmers' Horses.*—On the 22nd December, 1916, 44 horses were inoculated. Of these four horses died between the 15th and 18th February, viz., 55th and 58th days. The horses were discharged from experiment 30 days after inoculation. It is an interesting fact that they were the first horses to leave the stables at the expiration of this time, whilst the remaining ones only left the stable some time subsequently, and thus had been kept away from work for a longer period. It looks thus that the exposure of horses soon after immunization is a factor favourable for the development of acute Liver-atrophy. The diagnosis of these cases was made from description of symptoms and reports of two veterinary research officers who made an inspection. The mortality thus amounts to 9 per cent. (Serum 1719, virus 10436 Tz. 14th gen. and 10699 O. 176th gen.)

3. *Pietpotgietersrust.*—On the 4th December, 1916, 83 horses were inoculated, of these 15 died between the 24th of January and 21st February, viz., the 51st and 79th days. The number included one 4-year-old stallion, four mares, and ten geldings. The diagnosis was made from description of symptoms and report by a stock inspector. In three cases livers were submitted for histological examination and the diagnosis was confirmed. The mortality thus amounts to 18 per cent. (Serum 1717, virus 10451 Tz. 14th gen. and 10437 O. 183rd gen.—10452 Tz. 14th gen. and 10437 O. 183rd gen.)

4. *Warmbaths.*—On the 6th, 7th, and 17th November, 111 horses were immunized against horse-sickness. Of these 22 died between the 56th day and 104th day. The greatest number (16 horses) died between the 56th and 69th days, six horses died after this period. These horses comprised one stallion, nine mares, and twelve geldings, of various ages. The diagnosis was made from descriptions given and supported in some cases by inspection by a veterinary research officer. The mortality thus amounts to 20 per cent. (Serum 1717, virus 10215 Tz. 13th gen. and 10036 O. 176th gen.)

5. *Zoutpansberg.*—On the 1st, 13th, 27th, and 29th November, 1916, 293 horses were inoculated against horse-sickness. Of these 52 died, of which 29 deaths occurred between the 50th and 69th day; six before the 50th day, the earliest on the 39th day, and 17 after the 60th day; the latest case was observed 112 days after inoculation. The diagnosis was made from the descriptions given of the symptoms and from reports by the Government Veterinary Surgeon. The mortality thus amounts to 18 per cent. (Serum 1713, 1715, and 1717, virus 10215 Tz. 13th gen. and 10036 O. 176th gen., 10219 Tz. 13th gen. and 10036 O. 176th gen., 10451 Tz. 14th gen. and 10437 O. 183rd gen., 10452 Tz. 14th gen. and 10437 O. 183rd gen., 10436 Tz. 14th gen. and 10699 O. 176 gen.)

6. *Barberton.*—On the 25th October, 13th and 27th November, 1916, 63 horses were immunized against horse-sickness. Of this number 17 horses died and included two stallions, one mare, and 14 geldings, from four years upwards. Twelve deaths occurred between the 50th and 63rd day. After this date four occurred. The last case was noted on the 117th day. The diagnosis was made from descriptions of the symptoms and reports after inspection by a veterinary research officer. The presence of Haemoglobinuria was noted in four cases. All animals were worked since discharge, and, as they were allowed to run day and night they were exposed to the heavy rains of December. Most of the horses were cast Defence Force horses and

some came from the Orange Free State. The mortality thus amounts to 20 per cent. (Serum 1715, virus 10215 Tz. 13th gen. and 10036 O. 176th gen.—10219 Tz. 13th gen. and 10036 O. 176th gen.)

7. *Middelburg and Carolina*.—On the 7th, 15th, and 23rd November, 1916, and 4th and 9th December, 1916, 138 horses were immunized against horse-sickness, and of these 24 horses died. Of this number 22 died between the 54th and 70th days, one on the 73rd and one on the 104th day. This lot included one mare; the rest were geldings and varied in age from two years upwards. The greater number of horses were cast by the Defence Force, some came from the Orange Free State, some from the Cape Province, and some from the Transvaal high veld. The diagnosis was made from descriptions of the symptoms given and supported in some cases by the Government Veterinary Surgeon's inspection. The mortality thus amounts to 18 per cent. (Serum 1715, virus 10215 Tz. 13th gen. and 10036 O. 176th gen., 10219 Tz. 13th gen. and 10036 O. 176th gen., 10451 Tz. 14th gen. and 10437 O. 183rd gen., 10452 Tz. 14th gen. and 10437 O. 183rd gen.)

8. *Rustenburg*.—On the 28th, 29th, and 30th November, 1916, and 4th December, 1916, a total number of 280 horses was immunized against horse-sickness. Of this number 60 horses died and one was reported to have recovered; the mortality occurred between the 36th and 137th days. The greatest number died between the 51st and 70th days, viz., 33 horses; before this date five horses died and after this date 20. In two cases the date of death is not known. Amongst this lot of horses were 16 mares, and the rest were geldings. Their ages varied from three years upwards. The horses were obtained from the Defence Force and a number from the Orange Free State. All the horses that showed the disease were worked shortly after discharge and were running in the veld. Only in two cases it was stated that the horses had constantly been stabled. The mortality thus amounts to 21 per cent. (Serum 1715 and 1717, virus 10451 Tz. 14th gen. and 10437 O. 183rd gen., 10452 Tz. 14th gen. and 10437 O. 183rd gen.)

Summary and Conclusion.—Horses of the practice were immunized between October, 1916, and January, 1917, a total number of 1154 in various localities, viz., Johannesburg, Pretoria (Brits, De Wildt, Police, and Laboratory), Pietpotgietersrust, Warmbaths, Zoutpansberg, Barberton, Middelburg, Carolina, and Rustenburg. Amongst these 210 horses were reported to have died of acute Staggers and were compensated for viz., 18 per cent. With reference to serum and virus the mortality is as follows:—Serum 1713 = 11 per cent., 1715 = 19 per cent., 1717 = 18 per cent., 1719 = 9 per cent.; virus 10215 Tz. 13th gen. and 10036 O. 176th gen. = 6 per cent., 10219 Tz. 13th gen. and 10036 O. 176th gen. = 5 per cent., 10219 Tz. 13th gen. and 10437 O. 183rd gen. = 4 per cent., 10451 Tz. 14th gen. and 10437 O. 183rd gen. = 10 per cent., 10452 Tz. 14th gen. and 10437 O. 183rd gen. = 9 per cent., 10436 Tz. 14th gen. and 10699 O. 176th gen. = 9 per cent.

It is possible that in some instances the diagnosis was not correct; this, however, can only be the case in a few, since the disease is of such a nature that mistakes are not easily made. In a number of cases it was supported by histological examination of the liver sent in or by inspection by a Government veterinary surgeon or veterinary research officer. We have three figures to compare: the one from Onderstepoort with a total number of 1148 horses and 2 per cent.

mortality, that of the Defence Force of 1411 horses with approximately 4 to 5 per cent., and that of the districts with 1154 horses with 18 per cent. mortality. The discrepancy between the former two and the latter one is enormous. All horses were treated in the same manner as far as the immunization is concerned, and in particular there was no difference in the serum and virus used for the horses of the Defence Force and of the farmers. If there is any difference then it is in the treatment of the horses that commenced after the discharge from immunization. The horses of Onderstepoort and of the Defence Force were not used for work of any kind after discharge and were stabled for some weeks before they were put to work. The horses of the practice were in most cases, as the evidence showed, utilized for work immediately after discharge, and were exposed day and night to all climatic influences, particularly to rain during the succeeding period, so that many farmers connected the outbreak with the exposure to severe rain occurring at that time. It is thus evident from this experience that inoculated horses exposed after immunization to severe outside influence contract the disease to a much greater extent than horses that are well cared for, as was the case with the horses at Onderstepoort and of the Defence Force.

4. *Acute Liver-atrophy observed in non-immunized Horses in the Practice.*—Seven cases in all were brought to notice by the farmers and the description of the symptoms in each case is largely given in the phraseology of the owners. The first case observed in the practice was in a stallion about 16-17 years of age. He had been in the district (Louis Trichardt) for 10 years and was considered to be a salted horse, having contracted Horse-sickness during the first year after his arrival and in later years having had two relapses. During the summer season he had been in the worst parts of the district and accordingly his immunity had been put to the test. The horse was in good condition. In March this horse was noticed to be ill, somewhat dull; this was on a Thursday of the week; on Friday and Saturday he seemed as if blind; subsequently he was seen dashing his head forwards against the wall of the stable. On the first two days he was able to maintain his equilibrium, but on the last day he was rocking and swaying in the stable, constantly taking a dive forward so that the wall above the manger was smeared with blood; he eventually died on Sunday morning, thus having been ill for three days. The second case was observed in the Waterberg District. A five-year-old mare was purchased in October, 1916, from a dealer in Johannesburg; the horse was taken ill in the morning, shivering, pressed its head forwards, broke out of the stable blind, with head to the ground, the symptoms lasting two days. During the time of illness the horse did not eat or drink; eventually it recovered. The third case refers to an eight-year-old stallion, brought to the farm when young (Rustenburg District). When two years old he passed through a very acute attack of Horse-sickness. Two years later he had a mild relapse. During the Rebellion at the end of 1914 he was on commando, during which time he remained in the Northern Transvaal. He got an attack of Strangles and was returned to the owner in the Rustenburg District. At the beginning of January, 1917, the stallion was running with an inoculated and a salted mare; the inoculated mare subsequently died of Staggers on the 16th January, 1917. The stallion in question showed very acute symptoms of Staggers on the 10th March. He was found rushing about the veld blindly; with great

difficulty he was eventually caught and brought to a tobacco shed, where all possible means were tried to control the animal, all of no avail. He was striking out with front limbs and pushing forwards with great force, always turning in the one direction, viz., clockwise. He was sweating profusely; at first there was incontinence of urine, followed by haemoglobinuria. Finally he broke loose and rushed into fences and through bushes, etc. He finally landed in a fairly deep hole containing water, where he remained pushing with his head forwards into the side of the hole. He died the same night. The fourth case is a nine-year-old stallion, born in the Rustenburg District, and passed through an attack of Horse-sickness with a very severe Dikkop reaction when young, and was allowed to run day and night, but never showed a relapse. This horse was also out on commando during the Rebellion, when it contracted Strangles and was returned to the farm. It appears that this stallion also served the inoculated mare mentioned above and on the same day that the mare contracted Staggers. Two and a half months later (6th April, 1917) the stallion began to show acute symptoms of Staggers, lasting about three days and from which he died. The symptoms given by the owner were: running about constantly, sweating profusely, pushing forwards with great force, etc. It is of interest to note that the owner stated that the stallion only served that one mare in question and no other. The fifth case was in the Rustenburg District in a brood mare, eight years old, salted for Horse-sickness with both Dikkop and Dunkop. The mare was grass-fed, with an occasional grain feed, was in good condition, and had had five foals. She was very seldom ridden and then for about three or four miles at a walk. She was a tame animal and used to come to the house for a bit of bread or green food. On the day the disease was noticed she was at the house as usual and appeared in perfect health; about an hour later she was noticed walking around the boy, who was holding her on a headstall and riem. She broke out in profuse sweating round the ribs. She was cold to the touch and the breathing laboured. The mare got worse and appeared to go mad, she would under no circumstances back, but continually pressed forwards; she was not violent, only pressing constantly forwards. During the night the animal died. The sixth case was noticed in a stallion bought some years ago as a salted horse, and which had been for a number of years running day and night, both summer and winter. When this horse was noted ill it was put into a camp; during the night it forced its way through the barbed-wire fence, and in doing so lacerated itself terribly. It was found dead next day a considerable way from the camp. The seventh case refers to a salted horse in the Pretoria District. The diagnosis of Staggers was made by the Government veterinary surgeon of the district, who is well acquainted with the disease.

Conclusion.—It is evident from these communications that acute Liver-atrophy also occurred amongst non-immunized horses. There is, however, no doubt that it was present to a much higher degree in inoculated horses. It is, furthermore, a remarkable fact that most of the horses had a history behind them of having been salted. The cases in Rustenburg observed in a mare and stallion that were in contact with an immunized horse sounds almost like an infection. It is, apparently, only a coincidence, as similar cases would have come to our notice if such had been encountered elsewhere.

Pathological Anatomy of Acute Liver-atrophy.

The inspection of the cadaver may, in the absence of any anamnesis, suggest the cause of death. The lesions of the integument found are abrasions or superficial or deep wounds on the head, in particular of the frontal region, of the supra-orbital processes and zygomatic ridges of the nose, lips, chin, also gums and tongue. Rarer are lesions on the wings of the atlas, the point of the shoulder, elbows, hips, and hind legs. These lesions are found in horses that died in the stable. In horses that died in a paddock or in the field they are usually due to barbed-wire, and consist of scratches about the head, lacerated wounds on shoulder and forearm, also on the sides and hind legs. Skin lesions may, however, be completely absent in cases of more or less sudden death, where from the beginning the comatose stage was dominant. Wounds and haemorrhages of the tongue may be found. On section this organ has in some instances been noticed to be unusually yellow. The visible mucous membranes may show a yellow tinge, in particular the conjunctiva, but also that of the mouth; sometimes ecchymoses on the membrana nictitans are present. Gelatinous and even haemorrhagic infiltration may be present under the skin on various parts of the body due to traumatic influences during life. There are no abnormal contents in the pleural or abdominal cavities or an increase of liquid. Only in one instance was there an increase both in pleural and abdominal cavities. The serous lining generally is smooth and glistening and is frequently yellowish tinged. The lymph-glands sometimes show changes and are then oedematous, hyperaemic, or even haemorrhagic. This applies in particular to the subparotids and retropharyngeals, but also to the upper cervicals and in some cases to the mediastinal and the bronchial glands; they may also be found to be yellowish tinged. Hydropericardium is more or less pronounced, the quantity of liquid found varies from 20 to 400 c.c. The larger quantities 250 and more must be considered to be an abnormal finding, the smaller ones are normal. It is possible that the previous recovery from horse-sickness may be somehow connected with the large hydropericardium. The liquid in all freshly examined cases is clear, and yellow to brown in colour. The blood is usually coagulated, occasionally only partially; a dissolution sanguinis was also noted. Haemoglobinuria was then present. The myocardium as a rule shows changes; it may be opaque and even boiled like, the muscular fibres in many cases show a very extensive fatty degeneration. Ecchymoses are present in the right endocardium and sometimes very frequent in the left and on the epicardium. The endocardial lining and also the valves may occasionally show a yellow tinge. The lungs generally show a slight oedematous condition, sometimes more, sometimes less, pronounced, with a collection of froth in the trachea and accompanied by hyperaemia. In some instances haemorrhagic infarcts were noted, which apparently were of accidental nature. The pleura frequently has a slight yellowish tinge. An atrophy of the spleen occasionally is present, the pulp then appears dry, and the follicles are hardly or not at all visible; on the other hand hyperaemic conditions are also noted. Ecchymoses in the capsule are rarely found. Sometimes a slight yellow tinge of the capsule is noticeable. On microscopical examination frequently the presence of brown pigment is very conspicuous in the shape of grains and granules, reaching sometimes a considerable size. The main changes are found in the liver; it will be advisable to describe these fully. The size of the

organ may or may not be reduced. In this disease it has a tendency to be rather on the smaller side, at the same time it is reduced in its sagittal diameter; the edges appear sharp and flattened. The capsule is either smooth, or partially shrivelled. Fibrous patches and filaments on the cranial surface of the capsule are remnants of a Peri-hepatitis and are conspicuous in most South African horses and cannot be brought into any relation with this disease. The consistence of the parenchyma as a rule may be firm, more rarely it is softened or brittle. In most cases it is the colour that attracts the attention; it is light brown to light yellow, rarely dark. The capsule frequently appears pitted, and then the same picture is seen on section. By the naked eye, or better with a small power (6x), a meshwork can be recognized consisting of greyish meshes enclosing a darker small area, which, as a rule is sunken; it contains the central vein. The greyish meshes usually stand out as jagged ridges, encircling the lobule. Microscopical examination gives a typical picture. In the central portion of the lobule around the central vein there are as a rule no more intact liver cells to be seen. There are very rarely any intact liver cells left at all, and if so they are immediately bordering the central veins or the periphery; comparatively intact liver cells are more oftener found in the periphery. Lobules, in which no liver cells can be recognized, are frequent. Where the liver cells are absent, there is a homogenous substance full of yellow pigment and of a granular appearance. In this ground substance frequently nuclei are seen. They are either the endothelial cells of the capillaries or white corpuscles of the blood, or emigrated round cells. The endothelial cells frequently contain yellow brown pigment. As a rule the central portion of the lobule, and with it the capillaries, are filled with red corpuscles and are much distended. These are the blackish spots in the greyish meshes seen with the naked eye. There are sometimes also extravasations present, the red corpuscles occupying smaller or larger areas of the lobule. The comparatively intact liver cells, which can be recognized, contain fat globules; usually these globules are large. A Sudan or Scharlach stained section shows then an inner ring of fat droplets around the central vein and a larger outer one bordering the lobules. Sometimes also cells within the lobule contain these fat droplets. There seem to be transition forms from lobules, in which practically all cells show fatty degeneration to lobules, in which there is but little or no fat left. The former are very likely the initial stages of the fatty degeneration, the latter the final, the fat having disappeared again. The greyish jagged ridge of the meshwork is due to the formation of new bile-ducts and new liver cells. These cells are light coloured, large, and contain large vesicular nuclei. They are larger than any of the liver cells left over. Two kinds can be recognized: large (giant) cells, round, oval, or oblong, in which there are a number of nuclei arranged in the periphery or in some cases also grouped irregularly; and a second kind, in which the cells are differentiated, each cell containing but one nucleus, and these are grouped around a lumen. These phenomena must be interpreted as budding bile-ducts, and the process is thus one of regeneration. Indeed, in some cases an interlocking of bile-ducts and normal liver cell rows can be seen, and a transition from bile-ducts with a lumen to that of cell rows, running parallel to each other, can distinctly be made out. The fact of a fairly rapid recovery of a horse after most alarming symptoms seem to point to the possibility of a regeneration of liver tissue to which budding bile-ducts apparently

lead. The findings in horses killed after recovery indeed point to the complete regeneration of parenchymatous tissue without formation of any reparative connective tissue. The proliferated bile-ducts and even the undifferentiated cells are, however, frequently also the subject of fatty degeneration, and we find in them fat vacuoles of different size. It is true that their destruction has never been observed to be as complete as that of the liver cells, but it is, nevertheless, so pronounced in some cases that the Sudan staining shows these cells packed with fat globules. A further fairly constant observation is the presence of round cells. They are most frequently and most constantly found in the septa. They belong in the greatest majority of cases to the small round cells of lymphocytic type, and they are usually scattered all over the septa, or collected in groups or streaks, and extend along the periphery of the lobule; not rarely they are also found scattered throughout the necrotic lobule, but particularly near the central vein. In some instances round cells of a larger type, corresponding to the polyblast type, with more cytoplasm, are present and even fibroblasts. In all cases pigment is present, in some very much indeed, so that already with the naked eye it can easily be detected. This pigment is either inside or outside the liver cells, outside in the detritus, apparently liberated from the destroyed cells, inside in relatively intact liver cells and in connective tissue cells of the septa. It is either in the form of a fine dust, or as fine granules, or in flakes, usually packed, and of a light brownish colour. At the same time in all cases much formaline deposit is present, particularly in the necrotic detritus. For this condition the necrosis is apparently responsible. The pigment gives in all instances a positive iron reaction, but not in all cases does all the pigment respond to this test, in some more, in some less, but there is always a surplus of brown pigment left. When an addition of pure concentrated sulphuric acid is made, then the yellow pigment turns into a bright orange colour, which in turn changes into a greenish colour, thus showing that it belongs to the blood-derived pigment. Gmelin's test for bile pigment failed in all instances where tried, and there was never any greenish pigment noticed, which is so typical in biliary fever, and which was also found in cases of ragwort poisoning where the capillary bile-ducts in the liver cells are frequently injected. A slight cirrhotic condition may be noted in some instances; these are considered to be coincidental and not necessarily connected with the disease. In the cases noted they were in no instance pronounced as to be considered of primary importance, being really overshadowed by the more acute lesions of the atrophy. Notwithstanding the failure to trace bile pigment in the liver tissue a general state of icterus is present in practically all cases. It can be detected in many living animals, more frequently on post-mortem. The icterus being general and revealing itself after cutting the skin as a yellowish discolouration of the subcutaneous and fat tissue, as a brownish staining of the muscles, as a distinct yellow colour of the serous membranes, of the mucosa of the respiratory organs and the intima of larger vessels, and even the lymph-glands. It is, perhaps, less conspicuous in the intestinal tract. It is particularly pronounced where an anaemic state is present. In our cases the anaemia, however, must primarily be considered to be accidental and of a post-haemorrhagic character, due to bleeding to which most of the serum horses had been subjected either at the time or previous to the attack.

The kidneys are usually the seat of lesions, particularly of a fatty

degeneration of the epithelial lining of the tubuli contorti, and to a lesser degree of the tubuli recti, showing itself as a yellowish opaque discolouration of the cortex. Frequently hyperaemia of the medulla, or both of medulla and cortex, or then an anaemic condition may be found. The supra renal glands in some cases show a distinct yellow cortex, due to the presence of much fat, frequently well pronounced in the zona glomerulosa. The pancreas shows discoloration in most cases.

Slight hyperaemia of the mucosa of the stomach and of the intestines is occasionally found, usually in patches; sometimes the mucosa is diffusely red or slate coloured. Usually no swelling is noted in any of these organs. Some mucous deposit on the mucosa may be present. The urinary bladder may be found empty or filled; normal urine of a turbid character or clear transparent urine may be present; it may also be brownish tinged and even haemoglobin stained. Petechiae on the mucosa may be seen. Occasionally an anaemic condition of the brain is present. Cholesteatomata that were met with must be considered to be accidental. The condition found in the bone-marrow is normal; haemorrhagic foci in a fatty medulla are found equally in all horses, healthy and sick ones. (Compare article on Pernicious Anaemia of Equines of South Africa, Third and Fourth Report, Director of Veterinary Research.) The muscles of the anterior and posterior extremities were examined in some instances, caput longum tricipitis and rectus femoris, and the presence of fatty degeneration in a number of fibres was shown; these fibres were usually separated by normal ones, only rarely were adjacent ones affected.

Pathological Anatomical Diagnosis.—General Icterus. Dissolutio sanguinis (rare). Liver: pigmentation and fatty degeneration and necrosis of the liver cells; stasis and extravasations. Emigration of round cells and regeneration of bile-ducts. Kidney: fatty degeneration of the tubuli contorti and recti; haemoglobinuria (rarely). Heart: fatty degeneration of the myocardium; ecchymoses of epi- and endocardium. Supra-renal glands: fatty degeneration of the cortex. Muscle: fatty degeneration.

Comparison with Human Liver-atrophy.—The pathological anatomical diagnosis of the liver lesions as given here for the horse correspond in every respect with those given before for man, as described in the literature. Accordingly we conclude that both diseases as far as the pathological anatomy is concerned are identical.

Symptomatology of Acute Liver-atrophy of the Horse.—The course of the disease is a very rapid one. In the majority of cases it lasts an average of twenty-four hours; but shorter periods of less than twenty-four hours, less than twelve hours, and less than six hours are met with. Thus it is possible that a stabled horse can be found dead in the morning, nothing having been amiss the previous night. A duration of forty-eight hours, or of three days, has been found in some which ended in death. Recovery in Onderstepoort was noticed in six cases, which occurred in a minimum of four days. Precursory symptoms were met with and were recognized when the horses were daily used and individually attended to, and thus under constant observation. These are a falling off in condition, which may or may not be connected with the disease at all. Horses which were inspanned and used for a drive were noticed to be sluggish, not to respond to the reins, to have an unsteady gait, and to stumble. Similar symptoms were seen in horses under the saddle. When in the stable they were seen listless and dull, not feeding, with head hanging or resting on

the manger; when outside they were seen hanging about the homestead, with a peculiar vacant expression of the face. Usually a yellowish conjunctiva can be observed. There is no fever present. The temperatures of most cases that occurred in our stables were daily recorded over a fairly long period, and there was never any rise indicative of the disease. Also during the attack there is only in rare cases a rise to be noted; a tendency to sub-normal is rather the rule. Only in one or two instances were the evening temperatures elevated contrary to all experience. Symptoms as occurred in horse 10094 (*vide* Appendix I) cannot be looked upon as precursory, viz., loss of condition, swaying gait, dragging of the limbs. They were due to the attack of Biliary fever from which the animal was recovering. Here the disease itself lasted a little longer than twenty-four hours. Symptoms of colic: uneasiness, kicking at abdomen, lying down frequently, profuse sweating, repeated micturition have been observed to precede the violent onset. The acute symptoms have a sudden onset. In our cases they developed during any time of the day, frequently also during the night. The evolution of the disease is very rapid; the characteristic violent symptoms are in most instances present from the very beginning; they increase rapidly in violence. We might distinguish a less violent course from a more violent one. In the former the horse is noted to be semi-conscious; it stands in the stable usually with the head hanging in a corner; occasionally it moves about, and in doing so brushes against the wall; where the loose-boxes are whitewashed, as is the case in our stables, its head at an early time shows marks of whitewash on the frontal regions. The horse may stand for a long time against the wall, or more frequently in a corner, its position is, as a rule, unphysiological, the front legs are crossed or spread apart, the hind legs are spread apart or touching. No food is partaken of and no water; the horse occasionally snatches at the bedding, keeps the food between the lips either without attempting to chew it, or only doing so at intervals. The violence of the symptoms may slightly increase, the pushing forwards becomes more prevalent, abrasions of the skin of the frontal region may occur by rubbing against the wall of the stable. The gait is slightly swaying or staggering.

In the more violent cases these symptoms may increase rapidly or they are present from the very onset. In this case the horse pushes its head against the wall or in the corner; this pushing forwards is so violently maintained that the horse, losing its support with the head, shoots along the side of the wall into the opposite corner, there continuing the same manoeuvre. In this pushing movement the head may take up all possible positions, it glides upwards, so that it rests with lips and teeth on the wall or downwards so that the neck rests on the wall, or sideways; the horse may have landed under the manger and kept there in a constrained position, so that the side of the neck supports the body, or the head is bent back on the side, the horse pushing with the shoulder. In this position the front legs glide under the body, the hind legs are stretched as if a start is made to pull an extra heavy weight, and it happens frequently that the front legs finally slip and the horse goes down into a sternal position, but it constantly attempts to get up again, which may or may not succeed. In the latter case the horse keeps its sternal position until it rolls over and falls to the side. When normal position is held, it is done so with much shuffling of the hind legs. So violently are these movements carried out or the position maintained, that the

impact breaks the skin, which becomes bruised, lacerated in various places, first above the eyes, at the nose, at the forehead, at the neck, at the crista faciei, the zygomatic ridge, the lips, the gums, the wings of the atlas, the point of the shoulders, and the hips. The wounds are soiled with whitewash of the stable wall (in our cases), the whole head, the neck, shoulder, and rump were frequently full of whitewash; the wounds are bleeding more or less profusely, and blood is smeared all along the walls of the stables, large blotches being present in the corners, on the mangers, on the bars, and on the door. A single glance at the horse or at the stable allows of a diagnosis. The horse may show real fits of madness; it knocks its head most violently about against the walls, the manger, the corners, the bars of the box, and it is only the solid fabrics of the stable that prevent it breaking to pieces, although the door on some occasions was forced open. When the sick horse is taken outside, or when it is running in a paddock, it soon finds itself landed in a fence. It moves along the fence, tearing its skin to pieces on the barbed-wire, tangling itself up in a corner of the fence, breaking down the wire, and, perhaps, finally pushing through it. When taken ill in a camp, where the obstacles are not near by, the horse keeps on moving, or, rather, staggering, in a line or in a circle or all over the place, always forwards where the head points to, until it accidentally falls. It may also find its way into trees, bushes, or jumps on wagons, or walks into houses, etc. These violent symptoms, which may be described as the stadium excitationis, are succeeded by a semi or a complete coma. The horse then goes down, mostly on its side with stretched-out extremities. At the beginning it is able to rise again; later the attempts are fruitless, and the final coma overtakes the animal.

Symptoms of dyspnoea are sometimes alarmingly pronounced during this stage. They are more of an accidental nature: when the horse pushes its head so violently against the bars of the loose-box that the point of the nose cuts between two bars and the nostrils are half closed, causing stertorous breathing with all the bruits of an inspiratory dyspnoea; the same can be seen in a horse pressing its head into a corner or against the wall. Also when down and stretched out signs of dyspnoea may be present. These, as a rule, increase towards the end and become pronounced as expiratory dyspnoea with the formation of the hypochondric groove.

Nervous symptoms of a less violent nature were also noted. The somnolent to semi-conscious stage from the very beginning has already been mentioned: very little or no notice is taken of the surroundings and no food is partaken of; this lasts for one or two days in cases which go over into recovery. In this semi-conscious stage but little or no movements of the eyes are shown; they are staring or half closed. The settling of flies is usually not noticed by the horse, but sometimes it shakes them off all of a sudden. Twitchings of the lips are occasionally present, or the lower lips are drawn in against the incisors, and the upper lips upwards; the tongue is sometimes placed between the incisors. Champing of the teeth is occasionally present, also foaming at the mouth and rubbing the nose up and down the wall. Drawing of the head upwards and backwards, the vertebral column being bent convexly forwards has been noticed (*Opisthotonus*). Kicking against the abdomen, looking at the side, lifting one hind leg, stepping high with front legs when walking, sinking suddenly on to the front legs, are symptoms that were recorded. Yawning has been seen in some cases at short intervals. One of the symptoms

frequently noted is profuse perspiration, and sometimes the disease begins with it; sometimes matted hair draws attention to its previous presence. Sometimes these symptoms are present during the whole duration of the disease. At the beginning the pulse is normal, even when the most violent symptoms are present it still may be normal in frequency, quality, and rhythm. In other cases the rate is increased, it becomes soft and small, and finally is imperceptible. The impulse of the heart at that time is increased and its area extended. Micturition is either normal or its frequency is increased, being repeated at short intervals. The position for urination is occasionally taken up, and straining to urinate is then observed. The urine is mostly clear and of light colour, or in other cases it is dark; haemoglobinuria was rarely noted. Polyuria was occasionally seen. A chemical analysis was made in some instances. The presence of bile pigment was positive in all cases, but they were by no means conspicuous every time. The albumen test showed in two instances its presence, in one case only in normal traces. A test on the presence of amino acids was made in two instances; in one case they were found to be present in traces, in the second they represented the large amount of 11 per cent. of the total nitrogen found. It is most likely that it is chiefly Tyrosine. The presence of amino acids—Tyrosine—has been demonstrated in the human disease, and is considered there to be one of the typical symptoms. The presence of nitrogen as NH_3 was found higher than normal in one case.

Summary of Symptoms.—Symptoms of the illness include icterus; they develop into an acute stadium excitationis, which is succeeded by coma, and in the majority of cases by death. Occasionally the excitation stage may be absent, sopor is then the dominant symptom; the course is usually very rapid. In case of recovery the symptoms are usually less alarming and pass over in a few days.

Comparison with the human disease.—*Mutatis mutandis* the symptoms are the same: Icterus, stadium excitationis, stadium comatosum ending most frequently with death. There is accordingly no doubt that also the acute Liver-atrophy of horses from a clinical point of view can be brought into close analogy with the human disease. Since the acute Liver-atrophy of both, men and horses, is such a characteristic disease, both from a pathological-anatomical and clinical point of view, it is evident that the disease hitherto described under the same name in the Veterinary Pathology cannot be the true liver-atrophy. As stated before, I have no doubt that the disease hitherto described as such is one of the liver, but I am inclined to identify it with a parenchymatous Hepatitis of a less acute nature, resembling more the Senecio poisoning of horses, which will be described in this article.

Differential diagnosis.—The term Staggers is also applied in South Africa to horses suffering from Dunziekthe, a chronic disease of the liver that shows itself as a cirrhosis, starting usually from the central vein and involving finally the whole lobule, so that the normal parenchyma is atrophied and replaced by connective tissue. The course of this disease is, as a rule, a chronic one, lasting for weeks and months; in some cases, however, the symptoms develop very suddenly, and then it resembles the acute Liver-atrophy so much that a differential diagnosis is only possible on post-mortem.

Treatment.—An attempt was made in several instances, as detailed in the cases enumerated in the appendix. No definite results were obtained.

Table of Cases of Acute Liver-Atrophy observed in Onderstepoort.

No. of Horse.	Description.	Age.	Received from.	Number Received.	Passed H.S.	Treatment.	Serum.	Virus.	Result.	Diagnosis of Disease.	Days since Treatment	Treatment after Immunization.	Observation.
8762	Chestnut gelding.....	Aged.....	20.6.14—Silberman & Co.....	30	4	22.10.14	—	Tz. 9, 12, 13, and 14 gen.	Reaction.....	21.11.14	30	—	—
9432	Bay gelding.....	Aged.....	23.7.15—Suskin & Salkow.....	1	1	14.8.15	O. 1711	Tz. 12 gen.... O. 175 gen....	Reaction.....	26.10.15	73	—	—
9571	Bay gelding.....	Aged.....	23.8.15—Secretary of Defence...	150	97	7.9.15	O. 1711	Tz. 12 gen.... O. 175 gen....	No reaction....	(1) 15.11.15 (2) 10.7.17	69 O. 20 mths.	Hyperimmunized Vir. O. 178 gen.	Haemolytic.
9482	Bay gelding.....	6 years.....	23.8.15—Secretary of Defence...	150	97	7.9.15	O. 1711	Tz. 12 gen.... O. 175 gen....	Reaction.....	17.11.15	71	—	—
9364	Brown gelding.....	Aged.....	3.6.15—Goldstein Bros.....	19	5	1.6.15	O. 1711	Tz. 13 gen.... O. 175 gen....	Reaction.....	19.11.15	165	Hyperimmunized Vir. O. 178 gen.	—
9994	Brown gelding.....	Aged.....	9.12.15—Secretary of Defence...	90	85	18.12.15	O. 1712A	Tz. 12 gen.... O. 175 gen....	Reaction.....	18.2.16	62	Hyperimmunized Vir. O. 179 gen.	—
9962	Bay gelding.....	Aged.....	9.12.15—Secretary of Defence...	90	85	18.12.15	O. 1712A	Tz. 13 gen.... O. 175 gen....	Reaction.....	22.2.16	66	Hyperimmunized Vir. O. 179 gen.	—
10006	Brown gelding.....	Aged.....	9.12.15—Secretary of Defence...	90	85	18.12.15	O. 1711	Tz. 13 gen.... O. 175 gen....	Reaction.....	27.2.16	71	Hyperimmunized Vir. O. 179 gen.	Haemolytic.
9812	Chestnut gelding.....	Aged.....	27.11.15—Secretary of Defence...	32	21	24.1.16	Weekly Dipping	—	—	1.3.16	37	—	—
9989	Bay gelding.....	Aged.....	9.12.15—Secretary of Defence...	90	85	18.12.15	O. 1712	Tz. 13 gen.... O. 175 gen....	Reaction.....	5.3.16	78	Hyperimmunized Vir. O. 179 gen.	Haemolytic.
9949	Grey gelding.....	Aged.....	9.12.15—Secretary of Defence...	90	85	18.12.15	O. 1711	Tz. 13 gen.... O. 175 gen....	Reaction.....	18.3.16	91	—	—
9938	Chestnut gelding.....	Aged.....	9.12.15—Secretary of Defence...	90	85	18.12.15	O. 1711	Tz. 13 gen.... O. 175 gen....	Reaction.....	21.3.16	94	Hyperimmunized Vir. O. 179 gen.	Nuttalliosis.
9632	Grey gelding.....	Aged.....	28.8.15—Secretary of Defence...	100	78	4.1.16	O. 1711	O.P. 1 gen.... O. 175 gen....	Reaction.....	28.3.16	84	Hyperimmunized Vir. O. 179 gen.	—
9880	Chestnut gelding.....	7 years.....	4.12.15—Secretary of Defence...	18	13	24.1.16	Weekly Dipping	—	—	1.4.16	68	Immunized after recovery	—
10150	Grey gelding.....	7 years.....	3.2.16—Secretary of Defence...	105	96	8.2.16	O. 1713	Tz. 13 gen.... O. 175 gen....	Reaction.....	10.4.16	62	—	Nuttalliosis.
9884	Bay gelding.....	Aged.....	4.12.15—Secretary of Defence...	18	13	24.1.16	Weekly Dipping	—	—	11.4.16	78	—	—
10109	Bay gelding.....	Aged.....	3.2.16—Secretary of Defence...	105	96	8.2.16	O. 1711	Tz. 13 gen.... O. 175 gen....	Reaction.....	15.5.16	97	—	—
10094	Light grey gelding.....	Aged.....	3.2.16—Secretary of Defence...	105	96	8.2.16	O. 1713	Tz. 13 gen.... O. 175 gen....	Reaction.....	24.4.16	76	Hyperimmunized Vir. O. 180 gen.	Nuttalliosis; spiro- chaetosis.
9831	Chestnut gelding.....	Aged.....	27.11.15—Secretary of Defence...	32	21	24.1.16	Weekly Dipping	—	—	22.4.16	89	—	—
10159	Cream-coloured gelding...	7 years.....	3.2.16—Secretary of Defence...	105	96	8.2.16	O. 1712	Tz. 14 gen.... O. 175 gen....	Reaction.....	22.4.16	74	Hyperimmunized Vir. O. 180 gen.	Nuttalliosis.
10601	Brown stallion.....	5 years.....	8.9.16—Quinlan.....	5	5	12.9.16	O. 1712	Tz. 13 gen.... O. 175 gen....	Irreg. reaction..	18.10.16	36	—	—
10717	Dark brown gelding.....	Aged.....	11.11.16—Secretary of Defence...	37	31	15.11.16	O. 1718	Tz. 14 gen.... O. 176 gen....	Reaction.....	12.12.16	27	Hyperimmunized Vir. O. 184 gen.	Haemolytic.
10641	Bay gelding.....	8 years.....	20.9.16—Secretary of Defence...	47	41	17.10.16	O. 1715	Tz. 14 gen.... O. 176 gen....	Indef. reaction.	4.1.17	79	Hyperimmunized 184 gen. Vir.	—
10721	Dark brown gelding.....	Aged.....	11.11.16—Secretary of Defence...	37	31	15.11.16	O. 1714	Tz. 14 gen.... O. 176 gen....	Reaction.....	5.2.17	82	—	—
10209	Dark brown gelding.....	Aged.....	20.3.16—Secretary of Defence...	18	12	22.12.16	O. 1719	Tz. 14 gen.... O. 176 gen....	Reaction.....	26.2.17	66	—	—
10884	Bay gelding.....	8 years.....	15.12.16—Secretary of Defence...	73	42	2.1.17	O. 1715	Tz. 14 gen.... O. 176 gen....	Reaction.....	14.3.17	71	—	—
10831	Grey gelding.....	Aged.....	15.12.16—Secretary of Defence...	73	42	3.1.17	O. 1715	Tz. 14 gen.... O. 176 gen....	Reaction.....	24.3.17	80	—	—
10943	Bay gelding.....	Aged.....	21.12.16—Secretary of Defence...	27	22	26.2.17	O. 1713	Tz. 14 gen.... O. 176 gen....	Reaction.....	24.5.17	87	—	—
10854	Grey gelding.....	8 years.....	15.12.16—Secretary of Defence...	73	42	30.1.17	O. 1715	Tz. 14 gen.... O. 176 gen....	Reaction.....	4.4.17	64	—	Killed.
10814	Chestnut gelding.....	Aged.....	15.12.16—Secretary of Defence...	73	42	2.1.17	O. 1715	Tz. 14 gen.... O. 176 gen....	Reaction.....	28.3.17	85	—	—
11046	Bay gelding.....	Aged.....	29.3.17—Silberman & Co.....	21	5	10.4.17	O. 1715	—	—	17.6.17	68	—	Killed.



II.—PARENCHYMATOUS HEPATITIS.

(SENECIO POISONING.)

The disease Dunziekte in Natal was previously and is still connected with Ragwort (*Senecio spec.*) and is in Griqualand East even known by that name. Indeed all earlier authorities on the subject of Dunziekte, viz., Chase, Robertson, Verney, declared certain species of *Senecio*, viz., *Senecio burchelli*, *S. latifolius*, to be the cause of that disease, which they concluded to have produced artificially by feeding horses on the plants mentioned. In the *Cape Agricultural Journal* of May, 1906, Mr. Robertson refers to an experiment conducted by Chase who started on the 24th November by feeding a young horse with $1\frac{1}{2}$ to 2 ozs. of *Senecio burchelli* continuing this as a daily dose until the 6th December; on that date the animal refused to eat the plant in the forage, it was then daily balled with that amount. On 8th December it was noticed to be falling off in condition and continued to do so, though the appetite remained good. On 28th January the horse died, but unfortunately the post-mortem was rather delayed, and putrefaction had somewhat marked the pathological changes. The liver was bile stained throughout, hard, and indurated, and the other organs were normal. On section, distinct traces of new fibrous tissue were observed. Another feeding test was made with *Senecio latifolius*, which is reported here in Mr. Robertson's own words: "Horse No. 18, 29th December, fed on 4 ozs. bush *S. latifolius* daily and repeated until 2nd March, 1906, when the animal which had gradually been going off in condition and purging at intervals, showed symptoms of colic intermittently during the 3rd, 4th, and 5th. On the 6th the horse refused food for the first time, appeared stupid, pulse slower than normal, temperature unaltered, the mucous membrane of the eye (conjunctiva) showed signs of biliary staining, and the buccal membrane was somewhat dirty in colour. On the 9th colic symptoms again supervened, and the animal appeared much worse, breathing stertorous, eyes half closed, and head resting on manger, occasionally boring into a corner, evidently quite unconscious, no food or water taken. On the 10th March the animal died quietly, remaining standing almost to the last. Post-mortem: biliary staining of the connective tissue; the liver small, hard, and cutting with a harsh sound, very cirrhotic, hardly any healthy tissue left, the bands of fibrous tissue surrounding the healthy liver lobule." It appears from these two experiments that after prolonged feeding with two different species of *Senecio*, death was caused in one horse with apparently little warning, and in the second case a disease of very short duration, accompanied by symptoms of colic, icterus, and excitation, and somnolence and coma.

In 1912 experiments into the cause of Dunziekte were undertaken by the Division and continued until 1917. It was thought necessary first of all to clear up the point whether there is any connection with *Senecio* poisoning. A total number of 24 horses and one mule were utilized. (Details of which see in Appendix II.) Of these one horse (5772) did not consume any ragwort at all. It was turned out into a paddock in which plants of *Senecio latifolius* were very plentiful, but it was never observed that the horse ate any at all. At the conclusion of the experiment the ragwort was still present, whilst all the grass of the paddock had been eaten by the horse. This observation

coincides with those of farmers made in different parts of South Africa. The opinion that Ragwort is not or only rarely eaten by horses is thus justified. The 23 horses that consumed the Ragwort received it cut up and mixed with other foodstuffs. So long as the plant was not in too great a quantity, the horses ate it; when it was increased they refused it and apparently none relished it, although the quantity given was usually eaten. The attached list gives in a tabulated form the *resumé* of the feeding experiments, detailing the quantities eaten, the period over which it was fed, the average quantity eaten daily, and the interval death occurred after feeding ceased. It appears from this list, that of the smaller quantities the disease was produced in one instance, viz., after the feeding of 32 lb. in 50 days, or an average of 10 ozs. per day. Quantities fed up to 44 lb. did not produce the disease in all instances, although the daily average amounted to from 6 to 13 ozs., this quantity being fed over a period not exceeding 58 days. Quantities of 38 and 39 lb. produced the disease in daily average doses of 6 ozs. when fed for a period of over 100 days. It would thus appear that smaller quantities can produce the disease when fed for a sufficiently long period. This is brought out in the experiment where horses were fed with a total amount of 48 to 50 lb. in 125 days at a daily rate of 6 ozs. In the experiment in which the daily average amounted to 20 ozs. and more, the total reaching 106 and 131 lb., the disease developed after a shorter period, viz., in 78-79 days; only one horse (9451) that received a total amount of 48½ lb., on a daily average of 17½ ozs. died at an earlier time, viz., 44 days. One horse (10074) and the mule did not develop the lesions. It would thus appear that practically all horses are susceptible to Ragwort poison; a few are even highly susceptible if the feeding is continued long enough. The average daily quantity need not be large; if increased, the period of evolution is shortened. A very important feature has, however, been brought out: that death due to Ragwort takes place after the feeding has been discontinued for some time. The shorter periods of three and four days do not come into consideration, since these animals on the date of discontinuing must be considered to have been ill. We might even apply this explanation to the two cases that sickened after 13 days whilst they were going through Horse-sickness (8220 and 8225), but it is more difficult to understand in the deaths that occurred 20, 23, 43, and 96 days later. Some light is thrown on this observation by those made in one horse that was placed in an experiment and died of Horse-sickness on the 56th day, and by one horse that was killed on the 73rd day, in which cases both showed the typical liver lesions. It is thus clear that once a sufficient quantity of Ragwort has been consumed it produces a Hepatitis and that this Hepatitis can develop subsequently or continues to develop, although the feeding has been stopped. The longest interval between stopping of Ragwort feeding and death was 96 days, a remarkably long interval. The facts must partially be explained as cumulative poisoning and as a fixation of the toxin not necessarily in the liver, but attacking this organ subsequently. Some of the horses that had been fed on Ragwort were subsequently placed in a Horse-sickness experiment. In two instances immediately after stopping the feeding the horses were injected with Horse-sickness virus and serum, and whilst a reaction of Horse-sickness developed both showed symptoms of Ragwort poisoning. One of these horses showed the symptoms of Staggers, from which it recovered, and one

died. It is possible that Horse-sickness is partially responsible for the onset of the disease, although further experiments do not show that such need have been the case. Some of the horses were immunized subsequently to the discontinuation of feeding; one of these died of Horse-sickness and showed the typical lesions (this is the horse that died after 56 days), whilst three horses that died subsequently passed through the Horse-sickness without any further complications. Since death occurred equally in horses that were not immunized during the feeding period, it does not seem that Horse-sickness can be accused as accelerating the onset of the disease. Some of the horses had been immunized against Horse-sickness previous to the feeding, but no difference could be noted in the onset of the disease between these and the non-immunized ones. Two observations merit some special consideration, viz., the case of horse 8220 that recovered from an attack and horse 10774 that was killed and on post-mortem revealed lesions that might have been regenerative processes of liver-cells practically healed out. The former case should at least indicate that recoveries are possible once the cause has ceased to act.

Symptomatology of Senecio Poisoning in Horses.

The disease generally developed and took its course without any fever. Occasionally, however, disturbances of the temperature were present, showing themselves as slight exacerbations even during the time of feeding and as disturbances of short duration, but reaching no appreciable height. In some cases a slight fever was present during the course of other clinical symptoms. The onset of the clinical symptoms was not definite and not typical. Usually a loss of condition first became noticeable, and this sometimes before the horse could actually be called ill. The abdomen then showed a tucked-up appearance. The faeces were found dry in some instances and of abnormal colour and covered with mucus, or of abnormal soft consistence in others; the colour in some instances was distinctly yellow. A remarkable symptom in some cases was the appearance of colic. It was of a mild type and appeared during the course of the disease, showing itself as a general uneasiness, striking with hind legs towards the abdomen and pawing with the fore feet, an inclination to micturate and occasionally lying down, but no rolling was noticed. A fairly constant symptom was the discolouration of the membrana nictitans, which was found to be yellowish with a brownish tinge, and therefore described as orange yellow. Frequently ecchymoses were present, described sometimes as bright haemorrhagic spots. They were few in number in some instances or so frequent that they gave the membrane a blotchy appearance. Petechiae were in one instance also found on the septum nasi. Disturbances of the heart were drawing the attention almost with the onset of the first symptoms and they were usually pronounced in an alarming manner. The pulse frequently increased rapidly, the number of beats passed a hundred, and up to even 120 per minute were registered. It soon became weak and small in character, and finally almost or totally imperceptible. The impulse of the heart increased at an early period in intensity and extensity. It became visible at a distance in some cases; in most it could distinctly be felt, its area was extended backwards and upwards. In one case a disturbance in the heart rhythm was noticeable, a number of contractions at times following each other

unusually rapidly. A peculiar vibration was felt on the chestwall in some instances above the place of the heart impulse. The sounds were well pronounced and with a metallic timbre at the end; sometimes both were practically confluent to a single one. Abnormal bruits were heard as chafing and crackling in one instance. The beating of the aorta abdominalis could distinctly be felt in one instance all along the back as far as the os sacrum. The negative venous pulse was in some cases well pronounced, the blood-wave running back to the head, particularly when the horse stood with head hanging. The respiration was also altered in some respect. The number of respirations was not increased in some cases and only slightly in others. The type, however, usually changed, taking a pronounced abdominal character. In one instance the inspirations were unusually long followed by jerky short expirations. Towards the end and when the horse was down the respiration was stertorous. The micturition was not observed to show any definite anomaly. The urine showed abnormalities. It was either dark brown in colour to red, a haemoglobinuria being definitely diagnosed *intra vitam* in some cases. In some instances a test for bile pigment was made, when it was found to be increased. Albumen was present in some cases. Symptoms of the nervous system were present in most horses. The horse was dull and inattentive, sometimes it rested its head on the manger, at other times kept it hanging, and in some instances pushed it into a corner, resulting in bruising. One case showed acute but mild symptoms of Staggers as described in acute Liver-atrophy, from which it recovered. When taken outside, it could be seen that the gait of the sick horse was abnormal; the horse swayed or staggered, it stood with spread legs, it rocked forwards and backwards, or it pushed forwards, although never violently. A semi-consciousness was at times outspoken when the horse would take up food and keep it in its mouth without chewing it, the eyes being kept half closed. Tremor was noticed in the musculature of the shoulder and elsewhere in some horses. The course of the disease was usually a rapid one, lasting only a few days. It also happened that horses died suddenly. Unusual symptoms were in some instances the presence of swellings; in one horse the fossa temporalis was so filled that a Dikkop was suspected, and the absence of a specific infection could only be proved by subsequent inoculation; swelling of the chin and lips were also recorded. Also swelling of the hind legs was found in one case. The disease apparently had also taken a chronic turn in at least one horse. Apart from a general wasting no other clinical symptoms were noted. This was the case with horse 10896 which, on account of extreme poverty, was killed, and showed on post-mortem the typical liver lesions. The case is, however, also open to another interpretation, viz., the horse was killed before the acute symptoms had developed which might have done so at any date in the near future.

Summary and Comparison.—In the acute Ragwort poisoning a series of symptoms are noted, which allow of a diagnosis of a liver disease, viz., the jaundiced condition of the mucous membranes of the eye, the disturbance in the sensorium of the horse, viz., the slight pressing forward, the uncertainty of the gait, the semi-consciousness, which stand in a certain analogy to the symptoms found in acute Liver-atrophy and which they repeat in a milder degree.

To these symptoms may be added those of colic, loss of condition, and loss of appetite, the latter two being always present. We so obtain the picture as given by Hutyra and Marek for the acute Liver-atrophy of the horse. These symptoms are undoubtedly connected with a liver disease; in our case with parenchymatous Hepatitis. Acute Liver-atrophy is, however, nothing else but a very severe form of parenchymatous Hepatitis, so that the resemblance of the symptoms can be accounted for, which only differ in degree and which stand in a certain relation to the severity of the lesions found in the liver. To these symptoms have to be added those indicating the myo-degeneration of the heart, which are very alarming. They must be interpreted as being a direct result of the toxin contained in the *Senecio* plant, and not as a sequel of the liver disturbance. A similar explanation holds for the haematuria and haemoglobinuria that is occasionally observed.

The Pathological Anatomy of Senecio Poisoning.

The carcasses in most cases showed a somewhat emaciated condition that was more or less pronounced and stood, of course, in a certain relation to the duration of the disease. Decubitus was present in some animals on the head, shoulders, and hips. Swelling of the fossa temporalis was noted in one instance. Rigor was present in practically all cases that died a natural death. The visible mucous membranes frequently had a yellow tinge, the conjunctiva showed ecchymoses; also the presence of pallor was noted. The mucosa of the mouth in one instance was stated to have a brownish tinge. The subcutaneous tissue and also the fat present showed a yellow discolouration which had sometimes the colour of ochre. The absence of fat was, however, usually conspicuous. Extensive suggilations under the skin of some parts were found. The flesh had a brownish tinge and frequently a dry and opaque appearance. It was also found to be somewhat pale. The microscopical examination of the muscles showed a good number of fibres undergoing fatty degeneration; such fibres were evenly distributed, so that the cross-section of a muscle stained with Scharlach had a patchy appearance. The blood as a rule stained badly and had a brownish tinge; on the other hand it was also found to stain deeply. The mandibular, the parotid and upper cervical lymph-glands were usually found in normal condition, sometimes they were oedematous and also yellowish tinged, sometimes they were haemorrhagic and embedded in haemorrhagic connective tissue. The lower cervical and anterior mediastinal lymph-glands were frequently moist, enlarged, or even haemorrhagic with oedema of the supporting connective tissue. The broncho and posterior mediastinal lymph-glands were generally normal, but were also found enlarged, oedematous and haemorrhagically infiltrated. The submaxillary and parotid glands in some instances showed the yellow tinge, in some cases they also showed unusual moisture. The parotis was examined microscopically; patches of lobules showing fat globules in the epithelial lining were seen most frequently near a duct. The cells of the duct also showed fat globules. The submaxillary glands were also examined in one case. A number of lobules showed fat in the epithelial lining, in particular in the mixed lobules, so that in Scharlach stained sections the light transparent portion of the duct was surrounded by a red

ring of fatty cells. The thyroids were usually pale and also yellowish, rarely darker in colour. The situs viscerum was normal in all cases. No foreign contents in the peritoneum, or any increase in liquid were found. The serosa of the peritoneum and of the intestines were usually smooth and glistening, sometimes a yellowish or brownish tinge was present, also a bluish appearance in one instance was noted. Haemorrhagic suffusion in the lumbar regions and the regions of the arcus costarum was found in one instance. Diaphragm was in all cases found in its normal position with forward convexity; haemorrhagic infiltrations were found on the cranial surface. A small quantity of brownish-coloured liquid was occasionally found in the pleural cavity. The costal pleura as a rule was smooth and glistening. In the dorsal portion of the mediastinum extensive suggillations and less extensive in the caudal portion were found in one instance. The larynx was generally normal, but ecchymoses were also found on the epiglottis and the mucosa was then markedly hyperaemic. Some frothy liquid was escaping in most cases from the cut surface of the lungs. It was present with or without marked hyperaemia. The intima of the pulmonary vessels had frequently a yellow tinge. Froth was found in the bronchi, the mucosa of which was yellowish. The pleura had frequently a yellow tinge, ecchymoses were also seen. Emphysema was usually found in the apices and along the ventral border of the corpus pulmonis. The tracheal mucosa had frequently a pale or yellow appearance. Some froth was usually present; petechiae and ecchymoses were also found in some cases. An increased quantity of brownish clear liquid in the pericardium was the rule. It reached in one instance 500 c.c. A green tinge was noted in one horse, particularly distinct after shaking. The parietal serosa was smooth and glistening. Ochre yellow fat was found in the fibrosa and haemorrhagic suggillations at the base of the aorta. The right ventricle was frequently found in diastole and the left in systole. Ecchymoses on the epicardium were fairly frequent; they were at the base and along the longitudinal grooves; sometimes haemorrhagic suggillations and suffusions were noted. Ecchymoses were also found on the endocardium of both ventricles and in the muscoli papillares, also a slight gelatinous infiltration of the septum, of the valves, and a yellow colour of the whole endocardium were registered. The myocardium was usually opaque and yellowish coloured, soft; sometimes it had a patchy appearance due to the presence of lighter and darker areas. The colour in one instance was described as resembling terra cotta. The intima of the coronary arteries and of the vena magna were found normal. A suggillation in the wall of the vena magna was found in one instance. Intima of aorta and vessels were generally yellowish tinged. On microscopical examination lesions in the heart were found in all cases that succumbed to the Ragwort poisoning. The main feature was the presence of a fatty degeneration, which in many cases was so pronounced that the Scharlach-stained section offered a deeply red-coloured appearance to the naked eye. On microscopical examination this staining was found to be diffuse or patchy in distribution and not always of the same intensity in the different walls of the heart. The fat was distributed either in very fine granules, so that the fibres had a dusted appearance or in larger granules that obscured the cross section. In some instances the fibres were broken, the fat granules scattered about between the fragments of the fibres and round cells; large connective tissue cells

were present and a homogeneous substance. The round cells also were found filling the clefts between the fibres. Patches where fibres were absent were frequent and the deficiency in most of them was filled with connective tissue cells and round cells; fat also was noticed in Purkinje's fibres in some cases. Liver: the periportal glands as a rule were enlarged and moist. The organ was usually increased in size, the consistency usually tough. The borders were somewhat blunt. A distinct nutmeg appearance on section was present in most cases. The central veins were then distended, frequently surrounded by pigment; the peripheral portion of the lobules greyish to yellowish. Sometimes a grey meshwork could distinctly be made out, enclosing a reddish substance. In some cases the colour of the section was described as greenish brown, sometimes with red streaks and points in a yellowish meshwork, giving the organ the appearance of granite. The colour was also reddish brown. On microscopical examination two types of lesions were encountered, which differed by the presence or absence of proliferating bile-ducts and liver cells; the former type was in the majority. In most cases the central vein was enlarged and all the symptoms of a stasis were well developed; the lumen of the vein was distended as well as that of all the different affluent capillaries, sometimes so much so that the central portion of the lobule represented practically a blood pool or blood lagoon that occupied a smaller or larger area of the lobule. In some cases where the lesions of stasis were less developed the centre was found to consist of darker stained liver cells or portions of liver cell rows and surrounded by blood. In one instance the greater portion so occupied by blood showed in the Scharlach stained section the red stained fat cells of the periphery in striking contrast to the greenish blood pool of the centre. Usually, however, the central capillaries were partially distended and the liver cell rows between them were reduced to small strips, disconnected in some places so that fragments of liver cell rows were completely surrounded by blood corpuscles. In some lobules the lumen of the central vein of one case showed leucocytes, which were also scattered about freely between the liver cells. A slight thickening of the central vein was noticeable in some instances, the wall having a hyaline appearance. In one instance the fibrillar bundles of the walls were separated and formed a loose structure occupied by red blood corpuscles. Few lobules were noticed to contain intact liver cells; a disintegration or dissolution being present varying in different degree in the different cases and in the liver of the same animal and even in the same section. The unusual appearance was apart from the deformation of the liver cells caused by the blood stasis, due to the vacuolation, viz., fatty degeneration, the cells in the Haematoxyline Eosine section appearing pale transparent. According to the distribution of the degenerative processes the lobules offered different aspects. In some instances the lobules were fairly distinct, and the only disturbance present was due to the vacuolation of the liver cells. In other lobules the radiating picture still present was distorted, some of the cells in the rows were missing, and the periphery of the lobule was surrounded by a zone of liver cells with unusual large vacuoles. In other instances in the middle zone of the lobule were remnants of liver cells, with a transparent substance between them, and where liver cells were left they were out of shape. There were liver cells noted smaller than normal ones and in other places cells that were

on the contrary larger; some cells were deeply stained, some lighter, some had large nuclei, some small ones. Scharlach stained sections showed the vacuolation to be due to the presence of fat; such fat was practically present in all cases, very strongly pronounced in some and moderately in other instances. The distribution of the fatty degenerated cells also varied in different animals. Most of the cells were either in the middle or the peripheral zone of the lobule, surrounding the centre like a corona; but also within the lobule fat globules were present irregularly distributed. There was fat present in patches in some lobules, whilst others were free of it. The fat globules were either small and more evenly distributed or very large and then obscuring the rest of the cells. Also in the newly-formed bile-ducts fat globules were found. In one horse the newly formed bile-ducts and liver cells had undergone an almost complete fatty degeneration. The lobule presented in one instance the aspect of a radiating reticulum of fat cells, and the meshes contained blood. Also the endothelial cells of the capillaries were seen to contain few fat droplets. In some instances the destruction of cells in the lobules had advanced to a complete disappearance of the liver cells, their place being occupied by a homogeneous substance. This observation was limited to some lobules or part of lobules only: between them were lobules with less destruction. The septa in most cases were involved in the pathological process. With the exception of one or two cases a round cell infiltration was noticeable in various degrees. They were scattered about in some and forming clusters of different intensity in other cases. The septum thus frequently had a thickened appearance, which was due to the presence of these cells and also to some homogeneous transparent substance that permeated it. In other cases fibroblasts were distinctly forming bundles or strands; they were alone or mixed with fibrillar bundles and a homogeneous substance seemed to separate the bundles. It was also noted that the fibroblastic tissue extended into the periphery of the lobule and between the liver cell rows and atrophying the adjacent liver cells; in one case some of the lobules were entirely surrounded by connective tissue. Leucocytes were also found scattered about throughout the whole lobule in some cases. The presence of fibroblastic and fibrillar tissue was however in most cases associated with the presence of proliferating bile-ducts. These were formed by large pale stained cells with large vesicular nuclei; when they were in the septum, the form of a duct was distinct. Their presence helped to increase the thickness of the septa. In most cases the bile-ducts radiated into the periphery of the lobule and then surrounded it with a zone of light-coloured cells, distinctly contrasting with the rest. The course of the ducts was twisted, pushing the old liver cells to one side, so that the old dark-coloured liver cells and lighter coloured new ones were alongside of each other. In some cases a lumen in the budding bile-ducts was quite distinct; in others not. It was also noted that the cells of the newly-formed ducts arranged themselves in longitudinal rows, parallel to each other and parallel to an old liver cell row alongside. Bile-ducts and cell rows were also noticed to interlace with old cells, and new cells formed the continuation of all cell rows, whilst in one place new cells were within old cell rows. The impression was gained in many instances that the newly-formed cells replace the old ones and transition from bile-ducts into liver cell rows

could be made out without much difficulty by the different shading of the cells; the liver cells were dark coloured and the new cells were light coloured. The fibroblastic and fibrillar tissue were following the bile-duct formation and acted as a support to the proliferating cells. There was in one or two cases proliferation present without any increase of connective tissue; at the same time the interlacing with the liver cell rows was present. Only in two instances were there no proliferating bile-ducts present. The presence of pigment was conspicuous in most cases. Two kinds of pigment were present: green and brown ones. The green one was bile. It was seen in a few cases, but so distinctly that no doubt was possible. It filled the bile capillaries, forming a fine reticulum between the liver cells. In one case the larger bile-ducts were also gorged and the bile was present in form of flakes. The brown pigment was distributed all over the cells, both in intact cells and in remnants of such and in connective tissue cells. Very commonly it was in the cells bordering the central vein, so that the tract of the vein could be detected by the presence of these pigmented cells. Frequently the pigment was also in the liver cells bordering the septa and in the connective tissue cells of the septum. The pigment showed up best in Sudan stained sections, particularly the brown one. In such sections both fat and pigment were frequently found in the same cells. In one case the pigment was so frequent that the lobules had quite a yellow aspect. The lymphatic vessels of the caudal surface of the liver were distended and were standing out prominently in some instances. The pancreas was found to be of saffron colour in some cases. The splenic lymph glands were found occasionally enlarged and oedematous or infiltrated with blood. A slight tumor splenis was also registered. The capsule was smooth and tense. The pulp was found dark red in colour or dark brown. The consistence was soft or normal; trabeculae were not always distinct; the follicles mostly were not distinct. On microscopical examination in many cases light brown or dark brown pigment was found in the sinuses. In one horse a bright yellow pigment was present in the trabeculae. Atrophy of the lymphfollicles was also noted. The supra-renal glands were sometimes found enlarged, with a hyperaemic medulla and a yellowish cortex; sometimes they were very rich in blood. The cortex was almost in all cases found infiltrated with fat and sometimes to such a degree that the section stained with Scharlach revealed its presence by its diffuse bright red colour, so that the interpretation of a fatty degeneration in such cases was justified. The fat capsule of the kidney was usually poor in fat. The fibrous capsule stripped off easily. Frequent hyperaemia of the whole organ was pronounced. On section the cortex was frequently opaque, of brown yellowish colour, the consistence somewhat friable. The glomeruli were sometimes injected. A light yellow appearance of the cortex was also noted. The surface of the kidney was found mottled due to distension of the venae stellatae. On microscopical examination lesions in the kidney were present in most of the cases that succumbed to the Senecio poisoning. They consisted mainly in a fatty degeneration, which was present in various degrees, from a fine dusting of the cells to the complete filling of them with large globules. The tubuli contorti were the principal seats of the fatty degeneration, but the tubuli recti were also frequently crowded to a lesser degree, and in the intermediary zone the small tubules of Henle

were distinctly seen to contain fat droplets. The Haemotoxyline Eosine stained sections were pale transparent due to the vacuolation. Red corpuscles were occasionally found in the capsule. In many cases an exudate was present in Bowman's capsule of the glomeruli, pushing the tuft on one side and filling the lumen. In one instance the exudate showed drop formation, the drops reaching the size of red corpuscles up to 12μ in diameter. Similar exudate was also found in the tubuli contorti. Here sometimes the lining was desquamated, sticking to the casts. The nuclei were found to be pycnotic. Red corpuscles were found in the tubuli contorti in some cases. Glomeruli in various instances were found either with a thickened capsule or in the process of atrophying or completely atrophied, appearing as hyaline specks with a few pycnotic nuclei. In different cases there was a round cell infiltration in the adventitia of the vessels, the cells usually forming clusters. A hyaline degeneration of the vessel walls was seen in one case and the absence of tubuli in another case within such round cell collections. The bladder was found distended either with clear dark brown urine or with turbid urine. Chemical examination of the urine showed haemoglobin and methaemoglobin markedly present and bile pigment. Albumen was found to 0.5 per cent. The mucosa showed petechiae and yellow staining; the veins were injected; also extensive ecchymoses were found. The mesenteric lymph glands were sometimes found enlarged and oedematous or even haemorrhagic; so much so that in some cases liquid escaped on section. The size of the glands in one case was stated to reach that of a hen's egg. The mesentery was found diffusely hyperaemic; also an oedematous infiltration was present. Occasionally an unusual marked yellow colour of the tongue parenchyma was seen on cross sections. The pharynx was generally in normal condition, although frequently yellowishly discoloured. The mucosa of the guttural pouches showed in one instance injected vessels and ecchymoses; diffuse congestion was also noted. The oesophagus was always found in normal condition. In the stomach, food was either absent or, if present, only in small quantities; a dry blackish or greenish deposit was also found; sometimes the contents were liquid. Diffuse hyperaemia was occasionally present in the fundus with petechiae and bloodcoagula on the mucosa. The duodenum usually showed a yellowish deposit, but was sometimes slate coloured. In the jejunum haemorrhagic extravasations were seen; the mucosa was swollen in parts and bile stained, or slate coloured. The ileum was also found diffusely hyperaemic or with a slate discoloration. In the caecum and colon large patches of haemorrhagic extravasations and oedematous infiltration of submucosa were noted. The mucosa of the floating colon was found in some cases slightly thickened and diffusely reddened. The mucosa of the rectum was found to be covered with mucous deposit; the hard pellets present were covered with mucus. Brain: yellow staining of the pia mater was slightly pronounced, as also was the brain substance in rare cases. Bone marrow: gelatinous saffron yellow marrow and haemorrhagic foci were noted in some cases.

Conclusion.—The lesions found in the liver of Senecio poisoned horses must be interpreted as those of a parenchymatous Hepatitis. They were pronounced in various degrees, but nowhere to such an extent that they could be described as those of an acute Liver-atrophy, although in some horses occasionally patches of lobules were found

in which the picture of the acute atrophy was present in detail. Acute Liver-atrophy is, however, a form of acute Hepatitis and the difference between the two is only one of degree. The lesions in the heart must be described as those of myocarditis and fatty degeneration and correspond also to some extent to those found in acute Liver-atrophy. The main changes in the kidney were those of a fatty degeneration, in no way different to those found in acute Liver-atrophy. The presence of haemoglobinuria and haematuria showed itself in the casts found. An inflammatory condition was present in most cases indicated by the presence of round cell infiltration in the adventitia of the vessels. It is also possible that the atrophy of the glomeruli is a result of the Senecio poisoning. The fatty degeneration of the muscles and also the undoubted increase of fat in the supra renal glands were found to be present in the horses that died of acute Liver-atrophy. The presence of fat in the parotid and submaxillary glands should be a further indication that Senecio poisoning attacks practically all parenchymatous organs. In comparing thus the lesions found in Senecio poisoned horses with those that succumbed to Liver-atrophy, we find differences varying only in degree; the destruction of the liver tissue was dominant in the latter case, whilst the heart was more involved in the former; the rest of the lesions were more or less equally represented.

III.—THE CAUSE OF ACUTE LIVER-ATROPHY.

Discussion of the observations made and experimental results obtained and their interpretation as to the possible cause of the disease.

Considering the typical clinical symptoms observed during the course of this disease and the well-defined anatomical lesions found on autopsy, together with the fact that Staggers occurred in the greatest number of cases as a sequel to Horse-sickness after inoculation carried out with material obtained from horses not known to be suffering from Staggers, and after a period, that might be interpreted as that of incubation, one would be inclined to regard it to be a specific infectious disease, caused by a specific agency, a micro-organism. The spontaneous cases observed by us, which, in comparison to those seen after inoculation, are only a few, would then have been contracted naturally in whatever manner that is possible, whilst the cases observed in the immunized horses would have been transmitted by inoculation. The specific virus would have been present in the Horse-sickness serum or virus utilized for this purpose. The experience both in the laboratory and the practice showed that not all horses would be susceptible to this disease, and the susceptibility to a natural acquisition would still be smaller than to an artificial one; whilst the appearance of the disease in a horse again would still depend on further causes to which the horse was submitted during the period of incubation as was the case in the horses of the practice which died of Liver-atrophy far out of proportion to the inoculated horses of the laboratory and of the Defence Force. It would thus appear that the inoculation with the supposed specific organism would be followed by the outbreak of the disease only in a very limited number of cases, and that further contributing factors, such as the influence of environment, would help to cause outbreaks in a much greater number. To these contributing

causes the Horse-sickness itself may be added, through which practically all the immunized horses had to go, and the virus of which, as the transfusion experiments distinctly showed (*vide infra*) may still be present in the blood of some horses, at least at the time of Staggers. It would thus appear that the contributing causes are of as much importance as the specific cause itself. There is in this assumption nothing contrary to what is known to be the case in other specific diseases, primarily due to micro-organisms, although it must be admitted that a close parallel for such an extreme case as the one under consideration cannot be found.

The supposed infection would explain why we have not seen the disease previous to 1914, it having appeared only after that time. The fact that in the course of the acute Hepatitis no fever is noted does not speak against an infectious nature, although, with but rare exceptions, fever is a symptom of an infection. Parallels can be found. The assumption of an inoculable infective agency means that in our instances it must have been present in the blood of the animals from which the serum or virus had been obtained, and since these animals were not suffering from acute Liver-atrophy at the time the virus and serum were collected, it can be supposed that the infection was either present without causing any disease or that the animals that recovered from the disease acted as virus reservoirs. Whilst the former assumption is beyond control and the finding of it depends entirely on a lucky coincidence, the latter can be submitted to it. If we accept the presence of an infection in a horse not showing clinical symptoms we should expect that it would still be present when the symptoms have developed, at least at a very early stage, and we furthermore should expect to find it in the whole blood and perhaps also in the serum, and most likely in the diseased organs, the liver itself. Microscopical examination undertaken according to the usual methods did not reveal the presence of any organism, neither in the blood nor in the liver nor in any other organs of diseased animals. We should, therefore, have to assume that it belongs to the class of the ultravisable ones. We have accordingly to rely entirely on the results of inoculation experiments. The supposed micro-organism thus can be expected to be found (1) in the virus used to infect horses with Horse-sickness: (a) Tzaneen virus, (b) Ordinary virus, (c) other viruses; (2) in the serum used to control the virus injection; (3) in the whole blood of animals suffering from the disease; (4) in the serum of such animals; (5) in the liver of animals suffering from the disease; (6) in the blood of recovered animals; (7) in the serum of such animals.

Ad. (1) An observation with reference to the first contingency was made in one instance. A horse injected with a strain of Tzaneen virus, which strain serves as a first virus in the immunization of horses against Horse-sickness, developed the disease and died (details of this case are given in Appendix I, No. 8762). During the period under discussion a number of susceptible horses were injected with Tzaneen virus exclusively in order to test its virulency, their number of necessity being a comparatively restricted one. The one case (N. 8762) was the only one out of 52 horses that were injected with Tzaneen virus alone that recovered and remained sufficiently long under observation thereafter to exclude Staggers (at least three months). This corresponds to a mortality of approximately 2 per cent., a figure that we shall meet again in horses immunized simultaneously with serum. In this

immunization process Tzaneen virus was practically used every time as a first virus, therefore some suspicion might arise that it was indeed this Tzaneen virus which contained also the Staggers infection. This assumption falls, however, seeing that also horses not injected with Tzaneen virus contracted the disease. This refers to a horse that received "O." virus without showing any reaction at all, to a horse that received as a first virus one called Onderstepoort and as a second "O." to a horse that only received serum. Not one particular virus can thus be incriminated. The infection could perhaps be connected with certain horses, and might be present in the virus obtained from some horses and not from others. The horses amongst which Staggers was noted had been injected with Tzaneen virus of different generations originating from different horses. Thirty cases so analysed show that the virus utilized was obtained from 15 different horses. The maximum number of Staggers cases that can be found under one particular virus was five. This happened with two viruses which had been utilized on a large scale. In the case of "O." virus utilized in connection with Tzaneen virus and "O." serum, 27 outbreaks could be traced to five different horses. In one horse 15 cases were noted. This was the virus that had been mostly used. It would thus not appear that it is the virus of any particular horse that produced a greater number of Staggers cases.

Ad. (2) The number of cases observed in the different serum mixtures was as follows:—

In mixture 1711 were 9 cases out of 307 inoculated, 2.9 per cent.

In mixture 1712 were 3 cases out of 167 inoculated, 1.8 per cent.

In mixture 1712A were 2 cases out of 10 inoculated, 20 per cent.

In mixture 1713 were 3 cases out of 43 inoculated, 6.9 per cent.

In mixture 1714 was 1 case out of 104 inoculated, 0.9 per cent.

In mixture 1715 were 11 cases out of 440 inoculated, 2.3 per cent.

In mixture 1717 were no cases out of 207 inoculated.

In mixture 1718 was 1 case out of 30 inoculated, 3.3 per cent.

In mixture 1719 was 1 case out of 45 inoculated, 2.2 per cent.

The mixture 1711 consisted of the serum of 67 horses; the mixture 1712 consisted of the serum of 27 horses; the mixture 1712A consisted of the serum of 5 horses; the mixture 1713 consisted of the serum of 57 horses; the mixture 1714 consisted of the serum of 92 horses. In mixture 1714 was included the serum of 44 horses, hyperimmunized for the second time, that had previously been in serum mixture 1713. Mixture 1715 consisted of the serum of 120 horses. In this mixture was included the serum of 38 horses hyperimmunized for the third time that had previously been in mixture 1713, and of 41 horses that had been hyperimmunized for the second time that had previously been present in mixture 1714. Mixture 1717 consisted of the serum of 94 horses. In this mixture was included the serum of 28 horses, hyperimmunized for the third time, that had previously been in mixture 1714, and of 30 horses hyperimmunized for the second time, that had been previously in serum mixture 1715. Mixture 1718 consisted of serum of 100 horses. In this mixture was included the serum of 30 horses, hyperimmunized for the third time, that had previously been in mixture 1715, and 35 horses hyperimmunized for the second time and previously used in serum mixture 1717. Mixture 1719 consisted of the serum of 79

horses, and included 24 horses hyperimmunized for the third time that were present in 1717 serum and the serum of 29 horses after their second immunization that had been present in serum 1718.

By comparing the results reduced to percentage we notice that in the case of serum 1712A 20 per cent. of the horses injected showed Staggers, but the number of horses injected is so small that it must be ruled out, the coincidence being probably responsible for it. The same applies to a certain extent to serum 1713, after the injection of which about 7 per cent. of Staggers cases were noted; the total number injected, however, did not exceed 43. The remaining cases can be arranged as follows:—1717 serum with 0 per cent., 1714 serum with about 1 per cent., 1712 serum with about 1.5 per cent., 1715 and 1719 with about 2 per cent., and 1711 and 1718 with about 3 per cent. When we compare the composition of the serums, ruling out 1712 and 1713, we can see that there is practically very little difference in the incidence of Staggers, whether a serum mixture was composed of a small or large number of different horse-serums. This fact is also brought out in the horses inoculated in the practice where, however, the mortality was a much larger one. Here serum 1715 was utilized in 680 cases, amongst which 134 cases of Staggers were reported, corresponding to 19 per cent.; serum 1717 was used in the practice for 389 horses, amongst which 70 cases of Staggers were reported, corresponding to 18 per cent.; and serum 1713 for 9 horses, amongst which one case was noted, corresponding to 11 per cent. It is interesting to note that after injection of serum 1717 in practice a great number of Staggers cases were observed and none at the laboratory amongst a fairly large number of horses. It is thus evident that it is not a particular serum after the injection of which an unusual number of cases have occurred, and which therefore might be considered to be suspicious.

In the observations referring to Staggers in immunized horses the serum alone can of course not be made responsible, since all horses had also received virus. A test with serum alone was therefore required. This was carried out with serum 1715 and 1717. With serum 1715 alone 56 horses were injected in quantities as used for routine immunization. In one case a typical case of Staggers developed (horse 11046) from which the horse died (details, *vide* Appendix I). It showed the disease 68 days after the serum injection. With serum 1717 twenty susceptible horses were injected and no Staggers was noted thereafter.

The mortality of Staggers after the injection of serum would thus again be about 1 to 2 per cent. Since we have seen Staggers occur after injection of virus alone, in the case of Tzaneen virus in about the same percentage, no conclusions can be drawn as to the serum being responsible for this one case of Staggers noted. If we accept for argument's sake that a serum is a carrier of the theoretical specific organism, then this one experiment would only show that the susceptibility of horses inoculated under the conditions of the laboratory averages about 2 per cent.; under the conditions of the practice about 18 per cent. The fact that we have seen Staggers in horses injected with as little as 5 c.c. of blood (Horse-sickness virus) does not support the view that some hepatotoxic substance in the serum is responsible for the specific action.

Ad. (3) The blood of animals suffering from Staggers is the most likely place to find the specific virus if we maintain that such viruses enter the immunized horses either by means of Horse-sickness virus or serum. Particular attention was given to this contingency. The blood of nine different horses suffering from Staggers was infused into fourteen horses that were not immune against Horse-sickness. Eight of these nine horses had contracted Staggers subsequent to the immunization with Horse-sickness virus and serum, and one subsequent to an injection of serum alone. The longest interval between immunization and outbreak of Staggers was 77 days. The interesting observation was then made that the blood of the eight immunized horses produced horse-sickness in six of the twelve susceptible horses infused, four horses dying from it. The other six did not contract Horse-sickness, but three of them did so when subsequently tested and two died of uncomplicated pernicious Anaemia contracted from the same horse. One observation was, however, made which is of the utmost importance, viz., one horse (11057) went down 46 days subsequent to the infusion; it had to be destroyed, and on post-mortem showed the lesions of a *parenchymatous Hepatitis and of pernicious Anaemia*. A subsequent inoculation with blood of this horse (small quantities) gave a negative result as far as Liver-atrophy, but a positive one as far as pernicious Anaemia is concerned. Clinically, horse 11057 did not show symptoms of Staggers but pernicious Anaemia. The lesions of acute parenchymatous Hepatitis were well developed (*vide* Appendix III). The difference between acute Liver-atrophy and parenchymatous Hepatitis is only one of degree, the presence of a Hepatitis is therefore of the utmost importance. The absence of definite clinical symptoms is no unusual experience and does not speak against the case having not been one of Staggers; symptoms were also absent in a few horses that from the post-mortem lesions had to be diagnosed as Staggers (*vide* Appendix I). Here, then, would be a case of Staggers occurring some time after the infusion of blood, similar to those noted after the immunization of horses. This horse was, however, suffering at the same time from pernicious Anaemia, which fact complicates the interpretation of the lesions found. It is true that hitherto in a number of genuine cases of pernicious Anaemia no similar lesions were found by us, although liver lesions of a different nature are constantly present. But from the publications of other authors on pernicious Anaemia no support can be found to the view that parenchymatous Hepatitis forms a lesion of that disease. We must therefore consider this case as an accidental occurrence of the Liver-atrophy in a horse infected with pernicious Anaemia. A second case of acute Liver-atrophy was noted after infusion of blood of horse 11414 suffering from Staggers. Horse 11414 had been injected with Tzaneen virus (20 generation) and serum "O." 1715 on the 11th September, 1917. It died of Staggers on the 17th December, 1917, or 97 days after immunization. Horse 11499 was infused on the same date (17th December, 1917) and received 2500 c.c. blood. It developed Staggers 37 days later and died on the 23rd January, 1918. Horse 11487 that had received the same quantity of blood remained healthy.

In this second case no history of pernicious Anaemia is attached to the horses under consideration, and it appears that we have to do with a genuine case of Staggers after transfusion. This may be so.

On the other hand the possibility also holds good that a horse that would otherwise have contracted the disease spontaneously was accidentally selected for the transfusion.

However, two positive cases after transfusion out of fifteen cases represent a great percentage of positive results and look therefore very suspicious, and at least give cause to consider the result with an un-biassed mind.

Ad. (4). The serum of horses that had suffered from an attack of Staggers was injected into 72 horses, and did not cause the disease in any of the horses injected. This negative result can be interpreted in different ways. If we look at the serum to contain the specific organisms then it would mean that these 72 horses were not susceptible to the disease. It is, however, also possible that although the whole blood may be infective the specific organisms may not be present in the serum.

Ad. (5). Portions of liver of four horses that died from an acute attack of Staggers were made into an emulsion and eight horses were injected. Of these, three horses were immune to Horse-sickness. In two instances pernicious Anaemia was observed. In one case after an interval of 45 days the horse (10894) broke down to an attack of pernicious Anaemia, which it had contracted from a previous injection. The diagnosis pernicious Anaemia was made during life (*vide* details in Appendix III), and on post-mortem the lesions of this disease were present at the same time with those of a *parenchymatous Hepatitis*, which were, however, not so well pronounced as in case 11057 noted subsequent to Staggers blood transfusion. This occurrence is, however, of equally great importance, since it might be explained to be due to the injection of liver emulsion and to represent a case of Staggers. The argument as set out under (3) can be repeated here, except that a new possibility enters into consideration. If a specific agency should be present we would with a certain amount of right expect to find it in the organ in which the main lesions are found, viz., in the liver, and thus the chance of infectivity would be increased. After infusion of blood we noted two cases out of fifteen horses transfused, and after injection of liver emulsion one out of nine horses, or out of twenty-four horses injected with material obtained from Staggers horses in three cases positive results were obtained. This corresponds to a success of 12.5 per cent., and considering it in this light one would be inclined to accept the possibility of transmitting the disease by transfusion from a Staggers horse to a healthy one.

In this connection, however, another experience must be quoted. As will be shown later, experiments were made to produce Horse-sickness by infusion of blood from horses that had recovered from the disease into susceptible horses. Altogether the blood of ten horses was infused. Of these, one horse (11543) developed and died of Staggers 34 days after the infusion. It had been infused from horse 11278 that 56 days previously had been immunized with "O." and Tzaneen virus, but had never shown any sign of Staggers. In this case there would thus be a mortality of 10 per cent. from Staggers after infusion of immune horse blood. Considering this unusual experience in this light, one would also be justified in accepting the possibility of transmitting the disease by transfusion from a simple immune horse. The result of these experiments therefore can only be

interpreted that after transfusion of blood and injection of liver emulsion the disease may occur. But it is not the transfusion as such that causes it, otherwise we would have noted it long ago. We have hyperimmunized horses for over 12 years by infusion with quantities up to 10 litres and no Staggers was noted by us before 1914.

Ad. (6). The blood of recovered horses was used in four instances. Horse 9581 had recovered two years previously; 5000 c.c. of its blood were transfused into a susceptible horse (11366). Horse 9880 had recovered a year previously; 5000 c.c. of its blood were infused into susceptible horse 11372. Horse 10209 had recovered 222 days before 5000 c.c. of its blood were infused into horse 11367.

In no instance was there an outbreak of Staggers subsequently. It would thus appear that the blood of recovered horses does not produce Staggers. Since, however, the incidence of the disease, viz., the susceptibility under the condition of the laboratory, is only a small one—only about 2 per cent.—we might have missed the susceptible horses. On the other hand, large doses of virus, which would have been present in large quantities of blood, would break as a rule individual resistance, if such is not of a specific character. This was not the case in these instances.

Ad. (7). Serum of two recovered horses was injected: 26 horses received the serum of one horse and 57 horses the serum of a second horse. In no instance was there noted an outbreak of Staggers.

The explanation of the negative results of the serum of recovered horses may be given as in the case of serum from Staggers horses. The serum might not contain the specific virus, whilst the blood does; or, on the other hand, the 83 horses so injected were not susceptible to Staggers.

The negative results obtained from the infusion of blood and injection of serum of recovered horses would thus have to be interpreted, if we assume the virulency of Staggers blood, that a recovered animal is no longer infective, and this conclusion would make it very difficult to explain the transmission of the disease by virus or serum obtained from otherwise healthy horses that must be assumed to act as virus reservoirs.

If we recapitulate the observations made in connection with Staggers we find that it was noted under the following conditions:—

- (1) Spontaneously in horses of the laboratory, the Defence Force, and the practice.
- (2) After injection of Tzaneen virus.
- (3) After injection of Ordinary virus.
- (4) After injection of Tzaneen virus and "O." serum.
- (5) After injection of Tzaneen virus, "O." virus, and "O." serum.
- (6) After injection of Onderstepoort virus and "O." serum.
- (7) After injection of Onderstepoort and Bulawayo virus and "O." serum.
- (8) After injection of "O." serum alone.
- (9) After infusion of Staggers blood.
- (10) After infusion of liver emulsion of a Staggers horse.
- (11) After infusion of blood from a "T." and "O." immune horse.

The cases under (1) must be considered to be contracted in a natural way in whatever manner that may be. The remaining ones

may be considered to be due to the blood or serum injected that would have contained the theoretical organism. We assume that under the conditions of the laboratory the susceptibility of horses is a very restricted one; it only amounted to about 2 per cent. When we come now to consider the results in the practice, where the mortality was about 18 per cent., we find difficulty in explaining this discrepancy. It is quite true that in the evolution of an infective disease the condition of the animal, its state of resistance, its idiosyncrasy plays an important role, but it is most frequently the virus that is the determining factor, and we do not know of a parallel where one and the same virus should differ so much under two different sets of conditions as they varied here.

Taking all other things into consideration, absence of fever, impossibility of increasing incidence of disease by increased doses of virus, contracting the disease after various treatments as set forth above, the evidence is distinctly against the acceptance of an organism, although ultravisible, as we know them to cause disease in animals.

The main fact that has come out in connection with the occurrence of Staggers is the one that in the greatest number of cases it has been connected somehow with Horse-sickness. The greatest number of cases were observed in horses immunized against the disease, and in some spontaneous cases observed under the condition of the practice a previous history of Horse-sickness was attached to the horse, the horses were considered to be salted or had actually been seen recovering from Horse-sickness, whilst only in a few no such history is available. To these few belong the four horses that sickened spontaneously in Onderstepoort, one of which recovered (9580), and the one horse (11046) which sickened and died subsequent to a serum injection. In the latter case the serum, although one of Horse-sickness immune horses, could not have communicated Horse-sickness and therefore cannot be connected with this disease in the sense as accepted above. The blood of this one horse (11046) whilst suffering from Staggers did not cause Horse-sickness in any of the two infused horses, and this fact would speak against Staggers having been connected with Horse-sickness. Since, however, not all horses infused with Staggers blood contracted Horse-sickness, it is quite feasible to accept that in this one instance too it failed to convey the disease to the two horses infused. The one horse that contracted the disease spontaneously and recovered (9880) was later immunized for horse-sickness; it showed a typical reaction, with a slight Dikkop, and recovered. In this case the Horse-sickness must therefore be excluded as having been connected with the Staggers in the first instance. In all other cases no complete history is available, and the assumption that at one time previously they had been suffering from Horse-sickness cannot definitely be excluded, but this is not likely to have been the case for every horse. In support of the view that Staggers is somehow connected with Horse-sickness may be considered to be the result of the infusion of blood of horses suffering from Staggers into susceptible horses. The blood of eight Staggers horses was infused into 15 susceptible horses, and the blood of five horses produced Horse-sickness in six horses; the nine horses that escaped infection when later tested proved susceptible to horse-sickness, or died of pernicious Anaemia. The blood of one horse (11046) did not produce Horse-sickness; this horse has been dealt with above, it was not immunized with Horse-sickness as were the five horses that

gave positive results, and which had been immunized previously, viz., 66 and 59 days (10209), 71 and 64 days (10884), 80 and 71 days (10831), 64 and 58 days (10854), and 87 and 77 days (10943). The longest interval between injection of virus and death from Horse-sickness was thus 77 days. Thus a horse may up to 77 days after an injection of virus be infected with Horse-sickness. Not all horses so infused, and none of the two horses infused with liver emulsion, although susceptible, contracted Horse-sickness. The experience came quite as a surprise. It was looked upon almost as a dogma that a horse that recovered from horse-sickness no longer retained the virus in its system. Many experiments, giving negative results, had been made with small quantities of virus. In the light of the above experience with large quantities the negative results could be explained. The question is, therefore, whether all horses that recover from Horse-sickness retain the Horse-sickness virus in their system or only the horses that suffer from Staggers. A number of immunized horses were selected and their blood was infused in the quantity of 5000 c.c. into susceptible horses. In the case of one immune horse (11371) transfused six years after immunization and in the case of a second horse (10961) transfused four years after injection of Tzaneen and "O." virus, no results were obtained. The infused horses proved subsequently susceptible to Horse-sickness. In the case of a third and a fourth horse (11491 and 11492) transfused 71 days after injection of Tzaneen and "O." virus, respectively, no disease was noted. In the case of a fifth horse (11496) transfused 71 days after Tzaneen virus, no disease occurred; in the case of the sixth horse (11494) transfused 64 days after Tzaneen virus, no disease was noted. In the case of the seventh horse (11461), transfused 44 days after injection of Tzaneen and "O." virus, the disease was noted, the horse developing a Dikkop. In the case of the eighth horse (11436), transfused 48 days after Tzaneen virus injection, no Horse-sickness occurred, the infused horse proved later to be susceptible; and in the case of the ninth horse (11464), transfused 27 days after injection of Tzaneen and "O." virus, the disease occurred and the horse died of Horse-sickness. In the tenth case, after 56 days (11538 and 11543), no Horse-sickness followed. The summary of this result is thus that horses immunized with Tzaneen virus alone did not transmit Horse-sickness. Two horses immunized with both Tzaneen and "O." virus transmitted the disease, and the blood of the immune horses was thus still infective 27 and 51 days after virus injection. The other horses treated similarly did, however, not transmit the disease. The fact thus seems to be established that Horse-sickness virus can remain in an immunized horse for some time after its recovery. It must therefore be accepted that it is not the Staggers horse alone in which the Horse-sickness virus is found, but also in horses that do not succumb to Staggers. However, in our experience Staggers horses retained in a much greater number of cases and for a longer period the Horse-sickness virus than immune horses. This fact may be of importance. It is a further interesting observation that the horses contracting Horse-sickness from infusion showed in some instances the disease after an unusually long incubation period, and the disease itself, although with a typical curve, was of unusually long duration before death occurred, and out of six horses that contracted the disease two recovered. These observations would indicate that it is the first virus, the Tzaneen virus, that is still present

in the horse, the "O." virus having a much shorter incubation time, and being almost invariably fatal after a very short disease.

Staggers had not been seen previously to 1914, and horses were immunized by us long before that period. We must therefore dispose of the theory that Horse-sickness alone is responsible for Staggers as not tenable, but one fact is nevertheless quite evident, that if Horse-sickness is not the cause of Staggers it is at least an important factor in the outbreak of the disease. This follows from the regularity of the outbreaks noted after the various immunizations undertaken at Onderstepoort and at different other places. The toxic action of the Horse-sickness virus would have to be made responsible as a secondary and contributing factor.

In the experiments, in which blood or liver emulsion of Staggers horses was transfused into susceptible horses on three occasions, the appearance of pernicious Anaemia was noted. In two instances it was associated with Staggers. This may be but a coincidence. On the other hand here may be an association between the two diseases. If we compare the number of horses infused with Staggers blood that were infected with pernicious Anaemia at the same time, we notice that not all reacted with pernicious Anaemia; even in the case of horse 10854 that was undoubtedly infected with pernicious Anaemia, having produced the disease in 11057, it was not noted in all animals infused. We know from experience that horses that do not react to an injection of pernicious Anaemia virus are virus reservoirs, and their blood is infective for susceptible horses. If, therefore, Staggers was connected with pernicious Anaemia, not only the blood of horses suffering from Staggers, but also that of horses recovered from Staggers, should produce one or the other disease, if it is accepted that Staggers may be an atypical form of pernicious Anaemia. Furthermore, if we accept that Staggers was transmitted by serum or blood, then such serum injected by itself should have produced one or the other disease in a much greater percentage than it actually did. We cannot accept for a moment that all the horses injected did not react, because they were virus reservoirs. Indeed, subsequent events showed that several horses used previously for the serum injections contracted pernicious Anaemia when injected with such virus. We cannot, therefore, accept pernicious Anaemia as being the cause of Staggers. It may, however, like Horse-sickness, be a contributory one, and perhaps the two diseases together might act in concert in some instances. Both produce liver lesions. One disease may be constantly present in the horse (virus reservoir), the other one temporarily; whilst they are present they produce toxins, and these toxins act on the liver. These toxins can have a deferred effect, as is the case in Senecio poisoning, to which full consideration has been given in this paper. No proof, however, has been given that all Staggers horses were infected with pernicious Anaemia. On the contrary, the evidence goes against such an assumption.

There is still another way to explain the occurrence of Staggers. We consider it as a fact that both horses injected and not injected for Horse-sickness can contract the disease, although the disease occurs most frequently amongst the latter class. We consider the disease primarily to be one of the liver, due to some cause that tends to produce a parenchymatous Hepatitis. Generally speaking, this condition does not lead to the disease, except under a second, third,

or more influences. The second one being in most of our cases Horse-sickness, of which we know that the virus can remain in some of the immunized horses for a considerable length of time, during which period it continues to produce hepato-toxine, which finally upsets the already injured liver parenchyma. It is the primary cause we have to discuss. In this respect the *Senecio* poisoning of horses may give us a clue. We have established the extraordinary fact that horses fed for a time on *Senecio latifolius* may subsequently contract a disease, and this period in our experience in one instance (7299) was as long as 96 days. Its presence was also noted in one of the horses fed, which died of Horse-sickness, not showing clinical symptoms at the time, and one of the horses (8220), suffering at the time from Horse-sickness, actually showed symptoms which had to be diagnosed as acute Staggers, from which disease it recovered. The difference between acute Staggers and parenchymatous Hepatitis is one of degree, as was pointed out before; the two diseases merge one into another, and a limit between the two is only an arbitrary one. An acute Hepatitis resembling to a high degree Staggers can be produced by the plant poison of the *Senecio*, which poison only acts a considerable length of time after it has been consumed; likewise we may conclude that other plant poisons, not yet known to us, may act similarly. The Hepatitis so produced may or may not break out, and in the greater number of cases it does not break out, but when horses become infected with Horse-sickness, and perhaps pernicious Anaemia, they may break out through the action of the new toxine added to the one already present. The plant poison as already pointed out need not be and is most likely not Ragwort, a plant that is scarcely if ever eaten by horses, but it may be a plant or some plants with a similar poison. In this respect the climatic conditions for the last few years must be taken into consideration. In connection with the occurrence of other diseases, such as Lamziekte and Stijfziekte in cattle, we have shown that the rainfalls for many years have been abnormally low. Accordingly the pasture has in many respects been an abnormal one. There was generally a shortage of grass, hence the possibility that plants were eaten that otherwise are not eaten: plants that have similar toxic properties to *Senecio*. We thus come to the conclusion that the primary cause of Staggers is most likely a plant toxine that remains fixed in the liver cells for a considerable time. The third contributory cause, that which is responsible for the heavy mortality in the field, must be looked for in the way inoculated horses were treated. During a period of four weeks, during which they underwent the Horse-sickness reaction, these horses were stabled, and thus had a complete rest, not the least exercise having been allowed for them. At the conclusion of this time the horses in most instances were used and put to work, or they were allowed to run in the field day and night, exposed to sun, rain, and other influences, against which they all had been protected. Similar to what we are accustomed to see in the Haemoglobinaemia paralytica of horses, that is noted after a rest, this rest and subsequent sudden change may have been acting on a liver that was already otherwise poisoned and therefore broken down. Hence the difference in the mortality of the various batches of horses injected; in Onderstepoort only a small percentage, where the horses had been protected for a much longer period, a slightly higher one amongst the Defence Force horses, which were stabled at night and running during the day, but

not worked, and the horses in the field that practically all were worked and allowed to run day and night. In this respect one observation is of particular interest. A batch of farmers' horses were immunized at Onderstepoort; at the conclusion of the fourth week the horses commenced to be taken away. It was only amongst the horses that were the first to be removed that the disease was noted, and the first man who took his horses away lost two out of the three.

Of the three feasible explanations given as to the cause of the acute Liver-atrophy noticed by us, the one relating to a plant toxine as a primary cause, and to Horse-sickness and environment influences as contributory ones, is the only one that at the present state of our knowledge can be brought in line with all the facts observed in connection with the disease.

APPENDIX I.

CASES OF ACUTE LIVER-ATROPHY OBSERVED IN ONDERSTEEPOORT.

Case 1. Horse 8762. An aged chestnut gelding (purchased).

Anamnesis.—Arrived on the 22nd June, 1914, at Onderstepoort, malleined on the 8th July, 1914, and was passed as free from disease. It was utilized to test virus of the Tzaneen strain, receiving intrajugularly several injections, i.e. 5 c.c. of horse 8362, 14th generation, on the 30th July, 1914; 10 c.c. of the same virus on the 17th August, 1914; 10 c.c. of horse 7092, 12th generation, on the 14th September, 1914; 50 c.c. of horse 4376, 9th generation, on the 3rd October, 1914. No reactions were noted. 3 c.c. of horse 8361, 12th generation, on the 22nd October, 1914. This latter caused a reaction from the 6th to the 12th day, with a morning temperature of 100° F. on the first two days, 101° F. for the next two days, and 100° F. on the last two days. The evening temperature was never above 102° F. The fever was thus a very mild one. On the 7th November, 1914, 5 c.c. virus of horse 7284, 13th generation, was injected. No reaction was noted.

Symptoms.—On the 21st November, 1914, the horse showed symptoms of an unusual nature, resembling those of an acute brain disturbance. The temperature taken during the attack was subnormal, being below 96° F. The horse died the same night.

Post-mortem report.—The autopsy was made at 7 a.m. on the 22nd November, 1914. Rigor mortis was present. The condition was rather poor. Fresh traumatism was noted on the head. Natural openings showed no abnormalities. There were abrasions on the lower lip. The mucous membrane of the mouth was bluish. The flesh appeared normal. No fat was present in the subcutaneous tissue. Diaphragm convexly forwards. The tongue, pharynx, and oesophagus were normal. Larynx and cervical trachea contained a little yellowish liquid. The thyroids were pale on section. The salivary glands, the mandibular and retropharyngeal lymph glands showed nothing abnormal. The prepectoralis were hyperaemic, the bronchials and mediastinals were dark red in colour. The pericardium contained 100 c.c. clear liquid. Both ventricles were almost empty. The base of the heart was gelatinous. There were ecchymoses in the left endocardium, all the valves were normal. The endocardium of the right ventricle showed a yellowish discolouration. Nothing abnormal was noted in the myocardium. The thoracic trachea contained some foam. The intima of the aorta was smooth and glistening. Left and right lung were half collapsed. A few fibrous filaments were present on the pleura. On section hyperaemia and oedema were noted. Some froth was present in the bronchi. The interlobular tissue of dependent portions showed gelatinous infiltration. Intima of pulmonary artery and vein showed no abnormalities. The liver appeared to be small. On section the parenchyma was brownish in colour, fairly firm—numerous small calcareous nodules were scattered throughout. A fine reticulum could be recognized, consisting of greyish meshes including a brown substance; the former contained the septa. Fibrous patches and

filaments were present on the capsule, which in parts was shrivelled. The spleen measurements were 40×18 cm. The pulp was firm and somewhat dry; it appeared atrophic. The left and right kidney capsules were detached easily. On section reddish streaks were noted in the cortex. The consistence was somewhat firm. The pancreas and the supra renal glands showed nothing abnormal. The stomach was full of food; *gastrophilus* larvae were present. On the mucosa of the fundus were some superficial erosions. The duodenum showed no abnormalities. On the mucosa of the jejunum and ileum were hyperaemic patches, likewise in the caecum and colon. The mucosa of the rectum was somewhat hyperaemic. The bladder was distended, the urine of normal colour, and the mucosa showed some haemorrhages. Urethra was normal.

Intima of abdominal aorta was normal. In the branches of the anterior mesenteric artery was a thrombus. The bone marrow of the humerus was gelatinous; the cancellated substance of proximal end was red. Similar conditions were found in the femur. The brain showed no abnormality.

Pathological anatomical diagnosis.—Cachexia and general atrophy. Hydro-pericardium, ecchymoses of left endocardium; hyperaemia and oedema of lungs; atrophy and parenchymatous degeneration of the liver, atrophy of the spleen; slight enteritis; thrombosis of anterior mesenteric artery.

Microscopical examination.—The section had a somewhat mottled appearance [when examined under low power (6×)], showing a pale continuous reticulum, the meshes of which included darker patches. The central veins were distended and the walls appeared thickened in places. Relatively intact liver cells were present around the central vein and in the periphery of the lobules bordering the interlobular septa. These showed a slightly increased number of round cells of lymphocytic and polyblastic type, but no increase of fibrillar connective tissue. In the periphery were a number of large multinuclear cells; they differed from the adjoining liver cells by a paler cytoplasm and paler nuclei resembling those of the bile-ducts. Some were vacuolated and had lacerated outlines. The plasma of the liver cells showed different shades of staining. There was no cell continuity between centre and periphery of the lobule. The parenchyma cells between the two had practically all disappeared; only remnants of such could be made out and they were either vacuolated, containing large vacuoles, or they were lacerated, or only the bare outlines were left without any plasmatic contents. Their nuclei had either completely disappeared, or, when still present, had faded. The places formerly occupied by the liver cells contained either a homogeneous substance, or, and this is what took place to a greater extent, were occupied by distended capillaries, the endothelial walls forming a reticulum, the meshes being filled with red corpuscles. On a number of places the capillaries were broken and blood pools were formed. Here the only nucleated cells present were those of the white corpuscles. There was some pigment present: it showed the Berlin-blue reaction only to a limited extent. Much deposit was present, which could be cleared out with KHO (formaline). The portal veins were filled with blood. The nodules present were of parasitic type.

Pathological anatomical diagnosis.—Necrosis and fatty degeneration of the liver cells. Stasis. Pigment. Regeneration of bile-ducts. Emigration of round cells. Parasitic metastases.

Case 2. Horse 9432, an aged bay gelding (purchased).

Anamnesis.—Arrived on the 23rd July, 1915, at Onderstepoort; it was malleined on the 25th July, 1915, and passed into the stables as healthy. The horse was put in an immunization experiment, receiving intrajugularly on the 14th August, 1915, 5 c.c. virus of horse 8361, Tzaneen, 12th generation; on the 20th 50 c.c. serum "O." 1711 intrajugularly, and subcutaneously 20 c.c. virus of horse 9303 "O." 175th generation; on the 24th intrajugularly 275 c.c. serum "O." 1711. There was a slight reaction, typical of Horse-sickness, beginning on the 9th day and lasting to the 16th day, with two fever exacerbations on the 9th and 11th day. The horse was tested on its immunity on the 18th September, 1915, with 5 c.c. virus of horse 9302, "O." of the 175th generation, and no reaction developed. It was kept under further observation, during which time its temperature was normal.

Symptoms.—On the 26th October, 1915, at 7 a.m., the horse was found standing in a corner of its stall, pushing forwards, the front limbs stretched backwards. Wounds were noted about the head, particularly above the eyes. The horse was not quite conscious. 9 a.m.: The horse had fallen forwards, head and neck bent downwards and backwards under the body. It was released

from this abnormal position and placed in a loose-box. It received an injection of 15 c.c. of a 1 per cent. arecoline solution. 10 a.m.: The horse walked around in the box, and every now and then stopped, pushing forwards into a corner and standing in this position for some time, the position of the legs being abnormal. There was an increase of fresh bruises in the frontal region, and particularly on the supra orbital processes. 2 p.m.: The animal was down, lying on its side; it remained in this position during the rest of the day. 27th October, 1915. 7 a.m.: The animal was still found down, and was lifted, but it could not hold itself up, and immediately went down again, lying stretched out on its side. 10 a.m.: Another attempt was made to put the horse on its feet. This time it remained standing. It was, however, very weak, thin, and tucked up, semi-conscious, staggering to the side of the box and leaning the head on the manger. 2 p.m.: The animal was down again, lying stretched out. It died during the night.

Post-mortem report.—The condition was fair. Rigor mortis was not present. The integument showed wounds on the head. The visible mucous membranes of the natural openings were slightly yellowish. The flesh was somewhat dark. Subcutaneous tissue on several places contained gelatinous infiltrations. The diaphragm was convexly forwards. Pleural and peritoneal cavities showed no abnormal contents. The mandibular lymph glands were somewhat moist and hyperaemic, as well as the retropharyngeals and upper cervicals. Thyroids were normal. The salivary glands were slightly yellowish. Bronchial and mediastinal lymph-glands were somewhat rich in blood. Tongue on section had a pronounced yellowish tinge. Pericardium contained 200 c.c. dark-coloured fluid. Partially coagulated blood was present in the ventricles. The ostia were fully open. The vessels of the epicardium were injected, and numerous extravasations and ecchymoses were present. The endocardiums of both ventricles were haemoglobin stained, showing up in particular on the valves. The myocardium was somewhat pale and soft, apparently due to post-mortem changes. In the liver the post-mortem changes were rather advanced, gas formation and slate-coloured discolouration being present. The pancreas also showed post-mortem changes. The spleen measured 39×17 cm., pulp soft due to post-mortem changes. Supra renal glands showed no abnormalities. Kidneys: the capsule was easily detachable, parenchyma on section was pale, consistence somewhat soft. Stomach: the fundus showed a few hyperaemic patches and numerous *Gastrophilus* larvae. The mucosa of the small intestines were slate coloured; also that of the large intestines. The bladder was empty, its mucosa was normal. Nervous system: in the right ventricle of the brain a cholesteatoma (size of a walnut), and a smaller one in the left. Skeleton: Bone marrow of the humerus was fatty; that of the femur showed some haemorrhagic foci.

Pathological anatomical diagnosis.—Icterus; hydropericardium. Blood extravasations and ecchymoses on epicardium and left endocardium. Hyperaemia of both lungs. Cholesteatomata in ventricle of brain: post-mortem changes marked in liver, spleen, pancreas, and kidneys. Owing to the advanced post-mortem changes no microscopical examination was made of the liver.

Case 3. Horse 9571, an aged bay gelding, obtained from the Defence Force on the 23rd August, 1915.

Anamnesis.—It passed the mallein test on the 31st August, 1915, and was submitted to the Horse-sickness immunization on the 7th September, 1915, receiving intrajugularly 5 c.c. virus of horse 8361, Tzaneen, 12th generation; on the 13th September 50 c.c. serum "O." 1711 and 5 c.c. virus of horse 9302 "O." 175th generation, and on the 17th September 395 c.c. serum "O." 1711. It showed no reaction as a result of these injections. On the 5th October, 1915, it was tested on its immunity with 5 c.c. virus of horse 9302 "O." virus of the 175th generation, and again it showed no reaction. On the 25th November, 1915, it was hyperimmunized to the extent of 7500 c.c. blood of horse 9519 and 2500 c.c. of horse 9760 "O." virus of the 178th generation. On the 19th December, 1915, the serum was submitted to the haemolysis test and proved to be haemolytic.

1st Attack of Staggers.—

Symptoms.—On the 15th November, 1915, at 6 a.m., the horse was noticed to be sweating profusely, standing in its stall and pushing the head forwards against the bars in front; fresh wounds were noted about the head. It was then placed in a loose-box. At 7 a.m. it was noted that the lower lip was drawn tightly against the incisor teeth and the upper lip slightly upwards. Champing

of the jaws was present, the border of the lips were covered with a foamy white saliva. Symptoms of excitement were present in form of restlessness, walking round in the box. From time to time the horse stood with the head pressed against the wall. At 9 a.m. the animal, by pushing against the door, had broken out of the box and wandered about unnoticed. It was found standing on a paddock fence about $\frac{1}{4}$ mile from the box. It was brought back, and nothing unusual could be noticed about its gait. There was a profuse perspiration present. 9.30 a.m.: The horse received a subcutaneous injection of 1 per cent. arecoline solution. At 10.30 a.m. the salivation was profuse. The animal appeared to be hungry; it took up food but did not chew it. The horse moved frequently in a circle along the walls of the box and repeatedly rested, standing with the head against the walls, repeating the champing movements. Occasionally the head and neck were drawn slightly upwards and backwards and the ears backwards. Sometimes the head and neck bent so much backwards that the vertebral column was convexly forwards (Opisthotonus). The pulse showed no abnormal qualities, and the number was not increased. Respiration was slightly laboured. The animal had been seen to take up a position as if wanting to stale. Occasionally it was seen with the front legs crossed. 2 p.m.: The horse was found with food in its mouth, but not attempting to chew it. From time to time it was observed to snatch up small quantities of the bedding, holding it in its mouth. Salivation was less profuse. 3.30 p.m.: Five litres of blood were taken from the jugular vein as an attempt at curative treatment. 16th November, 1915. 9 a.m.: It is worthy of note that up to now the animal had not been observed to go down. It was somnolent, walking round in the box and often turning sharply round in the opposite direction. Unnatural positions were taken up, e.g. legs crossed, head and neck drawn backwards to the side, neck curved. There were whitewash marks above the eyes, on lips, and nostrils, as the animal had been brushing its head against the wall. Slight champing movements of the jaws were still noted. Lips and chin were pressed against the sides of the box, when standing quiet. 2 p.m.: The somnolence had further increased. The pulse was accelerated and small. There was an absence of movement of the eyes, which had a staring look. 4 p.m.: The horse was losing condition and looked tucked up. The urine passed during the day was yellowish and clear. On the 17th November, 1915, at 9.30 a.m., a slight improvement was noted. When led out, weakness in hindquarters was noticed, as well as a somewhat uncertain gait. The pulsations numbered 52; the respirations 16 per minute, they were slightly laboured. The conjunctiva showed a few superficial ecchymoses. The hair above the eyes was rubbed off. An unphysiological position was occasionally noticeable. When placed back in the box the horse was still walking round frequently, picking up bedding and chewing it. 11.30 a.m.: The animal appeared calmer, the somnolence not so marked; it was chewing a mouthful of bedding every now and then. 18th November, 1915. 10 a.m.: The horse showed great improvement. When entering the stable it showed attention. A natural position of body and limbs was taken up again. Feeding and drinking were as usual. Loss of condition was well pronounced. Swelling of eyelids was present. 19th November, 1915. Feeding and drinking normally. In order to keep this horse under constant observation it was placed in the transport stable. Since the date of discharge it had shown no signs of illness or abnormalities, viz., for a period of about twenty months, after which a second attack was noted, which, from the clinical aspect, was diagnosed as Staggers.

2nd Attack of Staggers.—

Symptoms.—10th July, 1917. This morning the horse was found down in the stable and brought then to a horse-box. It immediately took up the typical position of pushing the head in the corner. Later in the morning it went down again; it was raised on its legs and was able to keep its position. At times pushing forwards into the rails was noted. The mucosa of the lips was congested. The mucous membrane of the eyes had a yellowish tinge. The pupils were dilated. The surface temperature of the body was normal. The horse was then brought into the operating theatre and 2000 c.c. blood were removed. After the operation 100 grm. Dihydrogen Sodium Phosphate in 1000 c.c. physiological water were infused, whereupon the horse went down and was lying spread out on its side. Half an hour later the horse made an attempt to rise, but was unable to support itself. It was then brought to a loose-box, where it remained lying spread out on its side till 8.30 a.m. of the 11th July, 1917, when it died.

Post-mortem report.—The autopsy was made $1\frac{1}{2}$ hours after death. The condition was fair. Rigor mortis present. The integument was intact. The

tongue was hanging out. The pupils were distended. The anus was slightly open. The abdomen showed some distention. The blood was dark in colour, staining well. The flesh had a brownish hue. The fat had somewhat dark yellowish colour. On the outside of the parotid gland was found a blood coagulum (3×4 cm.). A haemorrhagic infiltration was present in the region of the throat about at the level of the thyroids and in front of these. The salivary glands showed no abnormalities. The tissue surrounding the trachea and oesophagus showed a slight oedematous infiltration. The mandibular, subparotid, and upper cervical lymph glands were moist, the surrounding supporting connective tissue was slightly oedematous. Left and right thyroids were pale. A thick deposit on the back of the tongue was present, which could be scraped off with the finger. The guttural pouches showed nothing unusual. The oesophagus was normal. The trachea and the larynx contained a little viscous liquid. The mucosa was normal. The pharynx contained a number of gastrophilus larvae, two were on the oral side of the epiglottis, and some pieces of grass were in the grooves of the tonsilla. The velum palatinum showed no abnormalities. The serosa of the intestines and of the peritoneum was smooth, moist, and glistening, having a slight yellowish tinge. The diaphragm was convexly forwards. On the caudal surface of the muscular portion of the diaphragm a small haemorrhagic infiltration was seen. Some fibrous filaments were present. The costal pleura had a slightly yellowish tinge. The bronchial and mediastinal lymph glands showed anthracosis.

Respiratory organs: The lungs were not collapsed. The left lung showed extensive emphysema in the apex, in the anterior portion of the main lobe, and along the ventral border. The pleura of the costal surface was bluish, slightly wrinkled, and fibrous filaments were found. The apex of right lung showed marked emphysema; it was also present in the interstia of the posterior border of the cardiac notch. The pulmonary pleura of the costal surface showed fibrous filaments and thickenings; the veins were injected. The tissue was fairly rich in blood; it was elastic. The main bronchus contained a little froth. The artery and vein showed nothing unusual. The right lung was rich in blood and the parenchyma dark in colour; on scraping off the cut surface a little liquid came off. The bronchus contained a little froth; its mucous membrane showed nothing unusual. The artery contained some coagulated blood; the intima was smooth. The vein showed no abnormalities. The mucosa of the thoracic trachea was smooth and glistening. The mucosa of the oesophagus was normal. The intima of the aorta and of the truncus brachiocephalicus was smooth and glistening. The anterior mediastinal and lower cervical lymph glands showed oedema and were embedded in an oedematous connective tissue. Pericardium: the fat present was yellowish. 300 c.c. clear yellow liquid were in the sac. The parietal serosa was smooth, moist, and glistening. Circulatory organs: Right ostium was open for four fingers, the left for three. Both ventricles were found in systole. Both ventricles and atriums were empty. The epicardium was smooth and contained a fair amount of fat in the grooves. The right ventricle showed a few ecchymoses on the septum, a suggestion on the musculi papillares of the septum and some infiltration of the parietal endocardium. The valvula tricuspidalis showed ecchymoses at the base. A suggestion 2×3 cm. was in the endocardium of the atrium. The foramen ovale was closed. The left endocardium had some suggestions in the septum and some infiltration in one of the musculi papillares and a haemorrhagic suffusion at the base of the valvula bicuspidalis. The coronary arteries showed the intima smooth. The left wall of the myocardium was 4 cm., the right 2 cm. in thickness, its colour was slightly greyish-brown, opaque, with a tinge of yellow. On section the septum was the same, and a yellowish slightly greenish tinge was pronounced. The consistence was somewhat soft. The periportal lymph glands were moist. The capsule of the oral surface of the liver was shrivelled, particularly in the ventral portions. A fibrous patch was noted on the oral surface of the right lobe. The capsule on the aboral surface was smooth and transparent. The edges were fairly sharp. The colour was bluish-brown. The left lobe on section was rich in blood and dark brown in colour; it also had a patchy appearance due to the presence of irregularly shaped small whitish areas. The central veins appeared filled. A hazy white lacework marking around the central vein was seen. The individual lobules were rich in blood. The pancreas was rich in blood. On section it was fairly moist, of light brown colour and fairly soft in consistence. The splenic lymph glands were slightly enlarged and moist. The spleen measured 40×21 cm. The capsule showed some fibrous filaments and some petechiae, particularly on the base; its colour was slightly bluish. On section the parenchyma was dark brown in colour. The trabeculae were fairly

distinct. The follicles were not visible. The consistence of the pulp was fairly solid, and could not be scraped off. The cortex of the supra renal gland was rich in blood. The adipose capsule of the right kidney contained much fat; the fibrous capsule was easily detached. On section the parenchyma had a pale brown colour, with slight stasis in the intermediate zone. The cortex had a slight greyish tinge. No glomeruli were discernible, and only a little striation. The consistence was slightly softer. The left kidney on section also had a light brown colour, and stasis was noted in the intermediate zone. The cortex was yellowish and slightly opaque, the consistence softer than usual. The stomach was full of solid foodstuff. The mucosa of the fundus was reddened in parts and showed a slight mucous deposit and a spiroptera tumour. A few gastrophilus larvae were present in the pars oesophagea and in the duodenum. The mucosa of the duodenum was slightly swollen and reddened. The mucosa of the jejunum and ileum was wrinkled and slightly yellow stained. The caecum and colon contained diffuse haemorrhagic infiltrations, and the mucous membrane was thickened. The mucosa of the floating colon was slightly thickened. In the rectum, faeces were present; the mucosa showed nothing unusual. Some fat was present in the mesentery. Intima of the abdominal aorta was smooth. In the arteria ileo-caeco-colica an aneurism was found, with nematode larvae and a little thrombotic substance. The urinary bladder contained some urine; the urine was very viscid and of a greenish-brown colour; the mucosa was slightly reddened in parts, particularly in the apex. The superficial inguinal lymph glands showed nothing unusual; no thrombi were present in the vena pudenda externa. The veins at the base of the brain were injected. The vessels of the pia were slightly injected. The left ventricle contained in the plexus chorioideus a small choleostoma. The ventricle was of usual size. The right ventricle also showed a small choleostoma: it was otherwise normal. The bone marrow of the femur was fatty, with a fairly large red focus; that of the humerus was also fatty, with a smaller red focus. The teeth were normal. Body weight 313 kg.; right lung 3.2 kg.; left lung 2.8 kg.; heart 3 kg.; liver 5.8 kg.; spleen 1.2 kg.; right and left kidney each 0.6 kg.; ratio of weight of liver to weight of body is 1:54. Urine examination: Total nitrogen .924 per cent., Ammonia .027 per cent. = 2.9 per cent. of total Nitrogen; Amino acids N .022 per cent. = 2.38 per cent. of total Nitrogen; Albumen .30 per cent.; bile pigments; slight positive reaction by Huperts test. Microscopical examination failed to show tyrosine. On testing with Fehling's solution a marked reduction was noticed, indicating a large amount of reducing substances, probably sugars. Nylanders reagent confirmed this. The phenylhydrazine test gave typical glucosazone crystals. The urine apparently contained a large amount of glucose.

Pathological anatomical diagnosis.—Icterus; Traumatic haemorrhages in pharyngeal region and diaphragm. Oedema of the lymph glands. Oedema and emphysema of the lungs; cloudy degeneration of the liver. Haemorrhages of the endocardium of both ventricles. Fatty degeneration of the myocardium. Perihepatitis fibrosa. Gastritis catarrhalis, enteritis haemorrhagica. Slight tumour splenis. Fatty degeneration of kidney. Fatty degeneration of suprarenal glands. Aneurisma parasitica. Gastrophilus larvae in stomach and pharynx. Choleostoma.

Microscopical examination.—Liver, left lobe: the lobular liver picture was present. All the vessels in the centre and in the periphery were injected, also the capillaries around the central vein were distended, and the thickness of the liver cell rows was reduced in these parts. There were two shades of liver cells, lighter ones in the peripheral portion of the lobules, and darker ones in the central portion, but the lighter ones were not so light as are newly formed bile-duct cells. In some places lighter patches of cells were traversed by tracts of darker ones, or both were irregularly mingled. In the central parts of the lobules vacuolation of the cells was distinct. Sudan stain showed here accumulation of fat globules of different size; there were also patches with fat cells irregularly distributed in the section. The staining of the liver cell nuclei was somewhat pale; the nuclei were showing up well. The interlobular septa were not thickened; there was no increase of connective tissue nor any cellular emigration. The bile-ducts appeared normal in staining and in number. Middle lobe: the stasis was much more pronounced than in the left lobe. In one portion of the section were a number of foci of different sizes, consisting mainly of eosinophiles; some of these foci were situated in the septa, others within the lobules. Some light brown pigment was present in the septa in some places. Sudan stain showed much

less fat than in the previous section and of a finer distribution. Right lobe. much blood was present in the central veins and the capillaries around them, leaving in places only a narrow rim of liver cell rows, between which the capillaries were much distended. In this central portion the capillary lumina were confluent and formed large pools, in which liver cell rows or individual liver cells stood out as islands; the cells were of darker colour and appeared narrow. Many of the cells were vacuolated, both of the lighter and darker coloured ones. The Sudan stain showed the fat finely distributed, only occasionally were found patches of cells containing larger globules. The fat was more in the intermediate portion of the lobule than in the centre or periphery, but the whole section had the appearance as if dotted with fat globules. Kidneys: some of the glomeruli were atrophied and hyaline. In a few of the tubuli recti the epithelial lining was desquamated and the cells conglutinated, the nuclei being pycnotic. Around an artery was a small cellular infiltration, and the lumen was filled with white corpuscles. In some places was some black amorphous substance placed between the tubules, and in some places distinctly within a conglomeration of red corpuscles. Round cell collections were also in the adventitia of vessels of the cortex. In some places the desquamated epithelium had the form of a hyaline cylinder with a few pycnotic nuclei. The tubuli recti and the tubuli contorti showed with Sudan stain a fine distribution of fat granules; the thinner portion of Henle's loop in particular stood out very distinctly in some instances. Supra-renal glands: in both glands all three zones of the cortex were filled with fat, so that the Sudan stain showed microscopically a diffuse staining of these parts. Lungs: the capillaries were fully engorged and the alveoli filled with a transparent substance that appeared somewhat pinkish after staining. In some of the alveoli, desquamated cells were present; the smaller bronchi also showed desquamated cells. In the lumina of some alveoli, usually in the centre was a black amorphous substance somewhat cloudy granular, the nature of which could not be made out (perhaps an artifact). There were also some dust cells present. Spleen: the sinuses were filled with red corpuscles, and much brown pigment was present between them. Lymph glands.—1. Mandibular glands: In some of the sinuses were rather large round cells with pale cytoplasm and nucleus, and between the cells was a homogeneous transparent pinkish stained substance. 2. Upper cervical lymph glands: The sinuses contained less cells, and empty spaces could be seen; the large round cells were also present. In some sinuses blood was present. Tonsillae: in some of the nodules the periphery contained an unusual amount of blood. Heart septum: in some places there were patches in which the muscular fibres were broken and formed small cubes, some of which were missing, so that here the section had a speckled appearance. There was an increase of leucocytes in these parts and also in the inter-fibrillar spaces of the adjacent parts. The pieces of the broken-up fibres showed no striation: they were homogeneous. Between some of them were small pools of red corpuscles and distended capillaries. The Sudan stain showed the whole section to be coloured, and under the microscope all fibres were seen to be crammed full with finely divided fat globules. Between the fibres were patches of connective tissue, apparently replacing former muscular fibres; some of these were included in the connective tissue, which was of a hyaline fairly bright pinkish colour, including some spindle-shaped nuclei. Left ventricle: here were fairly extensive haemorrhages in the muscular substance, and at one place were embedding the Purkin's fibres. In the substance of the muscle the presence of an increased number of leucocytes was conspicuous. The fibrous patches were also present—they were smaller than in the septum. The Sudan stain showed a patchy arrangement of fatty degeneration; the fibres were crammed full of globules. Muscles of the skeleton: occasionally a fibre took the Sudan stain.

Diagnosis.—Liver: fatty degeneration and stasis. Kidney: chronic Nephritis and fatty degeneration. Supra-renal glands: fatty degeneration. Oedema of the lymph glands. Heart: myocarditis haemorrhagica and fibro-blastica and fatty degeneration.

Epicrisis.—This horse had a history of two attacks of Staggers. It recovered from the first one and was then kept under constant observation, being utilized for transport work at the station. Twenty months later it developed a second attack of Staggers. No doubt as to the correctness of the diagnosis existed during life. Doubt only arose on post-mortem, and, in particular, after microscopical examination. The latter did not show the typical picture of an acute liver-atrophy. There was but little in the liver to account for the symptoms pointing to that organ being the seat of the

trouble. The principal lesions were found in the heart, but such lesions are usually not associated with Staggers symptoms as were noticed in this case. The small choleostoma found in the ventricle is not sufficient to explain these symptoms. The different shading of liver cells is not due to the budding bile-ducts as seen in liver-atrophy cases; indeed, no such bile-ducts were noted. A similar observation has been made in livers of horses that died of other diseases. The death of the horse was thus not due to acute liver-atrophy, and the discrepancy between symptoms and morbid changes cannot as yet be satisfactorily explained.

Case 4. Horse 9482. A six-year-old bay gelding, obtained from the Defence Force on the 23rd August, 1915.

Anamnesis.—The horse was malleined and passed into the stables as free from disease on the 26th August, 1915. On the 7th September, 1915, it was submitted to the immunization experiment, receiving intrajugularly 5 c.c. virus of horse 8361, Tzaneen, 12th generation; on the 13th 50 c.c. serum "O." 1711 and 5 c.c. virus of horse 9312, "O." 175th generation; and on the 17th 240 c.c. serum "O." 1711. It passed through a typical Horse-sickness reaction, beginning on the evening of the seventh day and lasting to the thirteenth day, with fever exacerbations on the ninth and eleventh day to 104° F. On the fourteenth day symptoms of dikkop were noticed, and the mucous membranes of the eye were slightly congested. On the seventeenth day a slight catarrhal mucous discharge of the nose was present. On the twenty-first day the symptoms of dikkop had disappeared. On the 5th October, 1915, the horse was tested on its immunity, 5 c.c. virus of horse 9302, "O." 175th generation being injected into the jugular vein. The horse was kept under further observation, showing constantly a normal temperature record.

Symptoms.—On the 17th November, 1915, in the morning the horse was found standing with the head pressed up against the corner of the stall, pushing forwards, indifferent to its surroundings, being semi-conscious. Fresh bruises and wounds were present in the frontal region. 9 a.m.: The horse when brought out of the stall pushed forwards with great force. It held some food in its mouth. Great difficulty was experienced in holding it back. The gait was somewhat uncertain. 10 a.m.: An infusion was made of 1000 c.c. blood into the jugular vein of horse 9401 which contracted Nuttallia Equi and subsequently died of Nephritis on the 4th December, 1915. Horse 9482 received a subcutaneous injection of 10 c.c. of a 1 per cent. arecoline solution. 11.30 a.m.: The wounds on the nose and above the eyes had increased in extent. The horse was vehemently pressing forwards, keeping its head lowered in a corner of the box. Marked perspiration on various parts of the body was noticed. No food was partaken of. 2 p.m.: The animal still pressed forwards, sometimes violently. Bruises and wounds in the region of the eyes and lips were even more pronounced. The horse knocked its head against the manger as if not conscious of such objects, and then walked round in the box rubbing the head along the sides of the box. 6 p.m.: Much the same sort of movement round the box was noticed, and when the horse came to a stop it pressed its head forwards against the wall. Occasionally it was seen standing with head under the manger. 10 p.m.: The horse was found standing in a corner of the box with shoulders pressed firmly up against the sides of the box, the neck bent downwards and head on the ground; every now and then it kicked with one hind limb towards the abdomen. The walls right round the box showed streaks and blotches of blood. Patches of the whitewash of the wall had been knocked off. The wooden partition showed whitewash marks brought there by the horse's head, extending from the ground to a height level with the horse's withers. The horse died during the night.

Post-mortem report.—The condition was fair. Rigor mortis had passed. The integument showed abrasions of the skin of the head. Mucous membranes of the natural openings and of the conjunctiva were slightly yellowish tinged. The subcutaneous tissue of the nasal and maxillary regions and the upper lip showed marked serous and haemorrhagic infiltrations. The costal pleura of the dorsum thoracis showed numerous subpleural haemorrhages. Diaphragm convexly forwards. Numerous fibrous filaments on the aboral surface were present. The vessels of the serosa intestinalis were injected. A few filaria equi were present in the abdominal cavity. Lymph glands: Mandibulars and retropharyngeals were normal; the thyroids likewise. The tongue showed haemorrhages in the parenchyma. Tonsillae appeared hyperaemic. Oesophagus was normal. The mucosa of the larynx was hyperaemic and petechiae were present.

Cervical and thoracic trachea were normal. The lungs were partially collapsed, a few subpleural ecchymoses were present. On section hyperaemia and oedema was noticed. A haemorrhagic infarct of the size of a nut was found in the left lung. Some foam was present in the bronchi. The arteries and veins were normal. Pericardium contained 100 c.c. of a reddish liquid. The ventricles were empty. The epicardium showed numerous ecchymoses. The right endocardium showed a few and the left one was sprinkled with numerous ecchymoses. The myocardium was pale and friable. The intima of thoracic and abdominal aorta was smooth and glistening. In the branches of the anterior mesenteric artery was found a thrombus and larval nematodes. The capsule of the liver was bluish and shrivelled and uneven in parts; the edges were sharp. The parenchyma had a mottled appearance; the consistence was somewhat soft. The central veins were visible and sunken. The pancreas showed no abnormalities. Spleen measured 39×16 cm., parenchyma was slightly soft, trabeculae were distinct. The supra-renal glands showed a hyperaemia of the medulla. The right kidney capsule was easily detached, the parenchyma appeared slightly hyperaemic. Left kidney showed similar conditions. The stomach was distended with food and gas. The fundus showed some patchy hyperaemia, gastrophilus larvae were present, and two small spiroptera tumours. Small intestines: mucosa of jejunum was slate-coloured, that of the ileum diffusely hyperaemic. Large intestines: mucosa of the caecum showed patchy hyperaemia; in the colon large hyperaemic patches were seen. Rectum showed no abnormalities. Bladder: some whitish mucus was present; numerous petechiae were on the mucosa. Bone marrow of the femur and humerus was fatty and haemorrhagic; red foci were present.

Pathological anatomical diagnosis.—Icterus; Hyperaemia of the serosa intestinalis; Hydropericardium; Ecchymoses of epicardium and endocardiums. Haemorrhagic infarct of left lung. Atrophy and parenchymatous degeneration of the liver. Hyperaemia of kidneys; hyperaemia of stomach and intestines. Parasites in stomach and intestines.

Microscopical examination.—The outlines of the liver lobules could be recognized by the presence of some round cells in the interlobular septa and by liver cells bordering them. These cells, however, were by no means intact: they appeared unusually large, in most cases without nuclei or then with faded ones; their outlines were lacerated, their plasma was honeycombed or areolated. The wall of the central vein appeared to be hyaline and thickened. In inner parts of the lobule no intact liver cells were left. There seemed to be a coalescent homogeneous ground substance in which were numerous vacuoles of all sizes and of different arrangements, the vacuoles being placed in clusters, in rosettes, or irregularly distributed. Sometimes pieces of liver cells could be recognized around a fading nucleus. In some place the endothelial cells of the vessels were distinct and stained well, in others they were absent. Between this homogeneous detritus red corpuscles were seen, so that one had the impression that the blood was filling up the room evacuated by the liver cells. The white corpuscles stained well, but were by no means abundant. There was some brown-yellowish pigment present, and a lot of formaline deposit. The pigment gave the Berlin and Turnbulls reaction. The round cells in the septa were mainly of lymphocytic type.

Diagnosis.—Fatty degeneration and necrosis of liver cells, Stasis, Pigment, Emigration of round cells.

Case 5. Horse 9364. An aged brown gelding, purchased on the 4th June, 1915.

Anamnesis.—Arrived at Onderstepoort on the 4th June, 1915. It was malleined and passed into the stables as free of disease. It was put into an immunization experiment for Horse-sickness, receiving on the 7th June, 1915, intrajugularly 5 c.c. virus of horse 8951, Tzaneen, 13th generation. On the 14th 100 c.c. serum "O." 1711 and 5 c.c. virus of horse 9302, "O.", 175th generation; and on the 18th, also intrajugularly, 210 c.c. serum "O." 1711. It passed through a typical Horse-sickness reaction of a mild nature, lasting from the 7th to the 16th day, with a maximum temperature of 103.8° F. on the 11th day. It was subsequently tested on its immunity on the 18th July, 1915, receiving an injection of 5 c.c. virus of horse 9302, "O." of the 175th generation. It proved to be immune. The horse was then submitted to hyper-immunization on the 17th and 18th November, receiving on each day an infusion of 5000 c.c. virus of horse 9757 "O.", 178th generation.

Symptoms.—On the 19th November, 1915, the morning temperature was noticed to be 101.2° F. At 7 a.m. the horse was wet with perspiration, standing with head pressed forwards into a corner; patches of blood stains were

seen in both corners and on the manger of the box. Fresh wounds were present about the head, eyes, nose, and lips. At times the animal pushed forwards with great force, standing with both front limbs under the body. Sometimes the front limbs slipped backwards while the animal pressed forwards; then again the hind limbs slipped backwards, with the result that the animal threatened to fall occasionally, but was nevertheless able to maintain the standing position with much shuffling of the legs. The horse was totally indifferent to its surroundings; it was semi-conscious. Great difficulty was experienced in removing the horse to a loose-box from the position taken up in the corner. 8.30 a.m.: When placed in the box the horse landed in a corner, assumed the following position: head and neck raised and drawn backwards (opisthotonus), chin and lips pressing against the wall, front limbs under the body, and hind limbs slightly stretched backwards, body pushing forwards, knocking about of the head. Occasionally the horse lost hold with its feet but regained the position. It was injected subcutaneously with 1 grain arecoline hydrate in 20 c.c. distilled water. 9.30 a.m.: The horse salivated and sweated profusely; it was leaning with the breast against the manger, the front limbs being placed irregularly under the body. The horse was not conscious of its surroundings and did not feed. 10.45 a.m.: A subcutaneous injection of 5 grains morphium hydrochloricum in 10 c.c. distilled water was made. 11.45 a.m.: The animal continued to press forwards with great violence, so that the nose jammed between the iron bars of the box, causing a stertorous breathing; shuffling of feet was frequent; knocking the head against the bars was repeated. The whitewash of the walls was knocked off by the horse rubbing its head against it, and blood streaks along the side of the box indicated its movements. It bled from the mouth and nostrils due to injuries. 12 noon: The animal was down. It received an injection per rectum of 2 ounces of chloral hydrate in mucilaginous solution. The solution was retained. 2 p.m.: The animal was lying stretched out, but every now and then struggling to regain the sternal position. During the afternoon three attempts were made to lift the animal on its legs, but it went down directly support was withdrawn. The animal died early in the night.

Post-mortem report.—The condition was fair. Rigor mortis had passed. Integument showed fresh decubitus on hips and head. Blood, flesh, and subcutaneous tissue showed post-mortem changes. Pleural cavities: Haemoglobin staining of the pleura was noticed. Diaphragm convexly forwards. Fibrous filaments on aboral surface. The peritoneal serosa was reddish discoloured. Mandibular, retro-pharyngeal, and lower cervical lymph glands were somewhat rich in blood. The tongue showed some abrasions on the apex. The mucosa of the larynx showed injection of the vessels. The mucosa of the pharynx was diffusely congested. The mucosa of the cervical trachea was diffusely reddened, and some petechiae were present. The thoracic trachea contained some froth. The left lung was partially collapsed; on section hyperaemia and oedema were noticeable. Froth was present in the bronchi, the mucosa of which was haemoglobin stained. The right lung was not collapsed; fibrous filaments were present on the pleura. On section hyperaemia, oedema, and a haemorrhagic infarct of the size of a nut were noted. The pericardium contained 400 c.c. reddish stained liquid. Right ventricle was dilated and contained partially coagulated blood. The endocardium was reddish stained. The left endocardium showed some ecchymoses. The myocardium was friable. The epicardium was haemoglobin stained. Thoracic and abdominal aorta showed no abnormalities; the branches of the anterior mesenteric artery contained a small thrombus. The liver showed post-mortem discoloration; on section it was greyish coloured, and gas bubbles were present. Pancreas was discoloured. Spleen measured 69×32 cm.; the pulp was softened due to post-mortem changes. Kidney capsule was easily detached; on section hyperaemia was noted. The fundus was slightly hyperaemic; some gastrophilus larvae were present. Small intestines showed a patchy hyperaemia of the mucosa. Large intestines: patchy hyperaemia and slate discoloration was present. The bladder showed mucoid contents, mucosa slightly hyperaemic. Bone marrow of the humerus and femur showed haemorrhagic foci.

Pathological anatomical diagnosis.—Hyperaemia and oedema of both lungs: Haemorrhagic infarct in right lung; hydropericardium, ecchymoses of left endocardium, slight gastro-enteritis; post-mortem changes advanced.

Owing to the advanced post-mortem changes no microscopical examination of the liver could be made.

Case 6. Horse 9994. An aged brown gelding received from the Defence Force on the 1st December, 1915.

Anamnesis.—Submitted on the 14th December, 1915, to the mallein test and passed as healthy. On the 18th December, 1915, subjected to the immunization against Horse-sickness. It was injected intrajugularly with 5 c.c. virus of horse 8361, Tzaneen, 12th generation; on the 24th 50 c.c. serum "O," 1712A, and 5 c.c. virus of horse 9302 "O," 175th generation; and on the 27th 405 c.c. serum "O," 1712A. It developed the typical Horse-sickness reaction to the first virus and another reaction to the second virus, lasting from the 7th to the 25th days, with a remission to normal on the 19th and 20th day. During the first period, on the 12th and 15th day the maximal record of fever was 104.8° F.; the morning temperature averaged about 101° F. In the second reaction the maximum reached 103° F.; the morning record was about 102° F. The symptoms of dikkop were noticed on the 14th and lasted to the 21st day; they were fairly pronounced and accompanied with ecchymoses of the conjunctiva. On the 28th January, 1916, this horse was submitted to hyperimmunization, receiving 5000 c.c. virus of horse 9916, "O," 179 generation, and 5000 c.c. virus of horse 9920, "O," 179th generation. No disturbance of temperature was noticed after this infusion.

Symptoms.—On the 18th February, 1916, at 10 a.m., the horse, which in the morning had been sent for exercise to the paddock, was brought back, and when examined showed bleeding wounds above the eyes; they must have been inflicted shortly before; bleeding from the nostrils was also noticed. The hair of the anterior part of the body was matted, showing signs of recent perspiration. The native in charge stated that the animal was noticed pushing forwards into the fence as if not able to see properly. The horse was placed in a loose box. It appeared dull, somnolent, and did not take much notice of its surroundings: it stood with its head pressed forwards into a corner of the box, every now and then changing the position of its limbs and pushing forwards with greater vigour than before. In this position it remained standing for some time. About 12 p.m. the animal was found leaning with its shoulder against the wall, the neck slightly turned to one side; the head showed further signs of fresh injury, and the wall above the manger was covered with patches of blood. When the animal lost its support against the wall it staggered in the box until it again found a wall to lean against. It was semi-comatose. Chloral hydrate was given per rectum in mucilaginous solution. 4 p.m.: The horse seemed quieter: it remained standing in one position for a long time. Since the beginning of the attack it had not been feeding. 8 p.m.: A sample of urine collected was yellowish and gave a negative result to Rosenbach's test for bile: it showed traces 1:10 by Jolly's albumen test. 19th February, 1916.—9 a.m.: The skin above the eyes was very much swollen and injured; the lips also were swollen. Head and body were covered with whitewash off the box wall. The horse was not feeding; it was semi-comatose and had a tucked-up appearance. Another injection of chloral hydrate in mucilaginous solution per rectum was given. 6 p.m.: The horse was found down lying spread out, fairly quiet, and not struggling. 20th February, 1916. 9 a.m.: There seemed to be an improvement. The horse was standing; it was again drinking and feeding slightly. The eyelids were greatly swollen and injured, and the eyes were half closed. The horse did not push forwards. The walls of the loose box showed blood marks all round from the ground to a height of about five feet. A slight diarrhoea was noticed. 10 a.m.: Swelling of eyelids and lips had increased. The horse walked into the walls, but stood fairly quiet in this position. A further injection of chloral hydrate was made per rectum. At 3 p.m. the animal went down and lay spread out exhausted and semi-comatose. Respiration was increased and laboured. At 7.30 p.m. the animal was found dead.

Post-mortem report.—The condition was fair. Integument: fresh wounds were present above the eyes. The conjunctiva showed petechiae. The flesh had a brownish tinge. The pleural cavities showed no abnormal contents. The diaphragm convexly forwards. A few fibrous filaments on peritoneal side. Peritoneal cavity showed no abnormalities. Mandibular and retro-pharyngeal lymph glands were normal. Thyroids likewise. The tongue was normal. A few *Gastrophilus* larvae were present in the pharynx. Respiratory organs: Larynx and trachea were normal. The left lung was collapsed; fibrous filaments on pleura, on section slight hyperaemia and oedema were noticeable. The bronchi showed no abnormalities. Right lung showed the pleura slightly yellowish, and fibrous filaments were present; on section hyperaemia and slight oedema were noted. The pericardium contained 50 c.c.

clear liquid. Circulatory organs: coagulated blood was present in both ventricles. The epicardium was slightly yellowish. Right endocardium showed no abnormalities; on the left were some petechiae and suggillations. The myocardium was pale and somewhat soft. Intima of thoracic and abdominal aorta slightly yellowish tinged. In the branches of the anterior mesenteric artery was a thrombus and nematode larvae were present. Liver: fibrous filaments present on the capsule. The parenchyma on section light yellowish brown. The lobules were indistinct; some brown yellow spots and streaks between a greyish substance. Some lobulation, however, could be recognized in some places. There was a greenish tint present, which was more distinct around the central vein, and which again was surrounded by a more whitish zone, thus giving the organ a mottled appearance. The septa could occasionally be well recognized. The consistence was slightly firm. Spleen measured 45×23 cm., parenchyma on section was dark red, trabeculae not quite distinct, consistence was somewhat soft. Kidneys: the capsule was easily detached; the parenchyma showed on section a yellowish tinge, and there was hyperaemia of the medulla. Stomach: fundus showed slight diffuse hyperaemia. Small intestines: diffuse hyperaemia and some mucous deposit. Large intestines: a diffuse hyperaemia was present; rectum showed a patchy hyperaemia. Bladder was distended with turbid urine; the mucosa was normal. Examination of urine: viscous, specific gravity 1031; albumen slight; acetone bodies slight; bile pigment present; glucose absent. Brain pale. Bone marrow of humerus and femur contained haemorrhagic foci.

Pathological anatomical diagnosis.—Slight icterus; slight oedema and hyperaemia of lungs; ecchymoses and petechiae of left endocardium; slight cirrhosis, fatty and parenchymatous degeneration of the liver; pigmentation; tumor splenis, hyperaemia of kidneys; slight hyperaemia of stomach and of small and large intestines.

Microscopical examination.—There was a slight cirrhotic liver. The septa stood out fairly prominently, but did not circumscribe the whole lobule; the thickening was, so to say, limited to the corners between the lobules. Here fibrillar tissue was present in fairly coarse strands. In the periphery of the lobule the cells were relatively the least injured. They possessed a paler cytoplasm and a large pale nucleus; they were, as a rule, concentrically arranged with a lumen in the middle. These were undoubtedly newly formed bile-ducts: they were crowded at some places. There were also multinuclear cells present not yet differentiated. On different places some of the newly formed bile-duct cells were honeycombed and vacuolated. The walls of the central vein appeared hyaline and thickened. There were practically no intact liver cells. They were found in all stages of disintegration. Some of them were increased in size, vacuolated or honeycombed, some consisted of a homogeneous substance; empty spaces were left where cells previously had been or only traces of plasma. Only nuclei were sometimes left. The nuclei themselves were faded or vanishing. The capillaries contained blood. The nuclei of the endothelial cells were distinct in most places. There was a yellow brown pigment present mainly in the plasma of the endothelial cells; it was also frequent in the septa. About half of the pigment gave the Berlin blue and Turnbull's reaction.

Diagnosis.—Fatty degeneration and necrosis of liver cells; slight cirrhosis. Regeneration of bile-ducts. Pigment. Stasis.

Case 7. Horse 9962. An aged bay gelding, received on the 9th December, 1915, from the Defence Force.

Anamnesis.—On the 14th December it was submitted to the mallein test and was passed as healthy. On the 18th December, 1915, it was subjected to the immunization, receiving intrajugularly 5 c.c. virus of horse 8951, Tzaneen, 13th generation; on the 24th, 50 c.c. serum "O" 1712 and 5 c.c. virus of horse 9302 "O" 175th generation; and on the 27th intrajugularly 430 c.c. serum "O" 1712. A typical reaction ensued from the 9th day, lasting to the 20th day, with an average evening temperature of 103.4° F., having on two occasions exacerbations to 105° F. Symptoms of Dikkop appeared on the eighteenth day, but were only slightly pronounced. They disappeared again on the twenty-fourth day. The pulse frequency during the period of reaction averaged from 44 to 52. This horse was submitted to hyperimmunization on the 23rd January, 1916, and received 5000 c.c. virus of horse 9908 and 5000 c.c. virus of horse 9906 "O" 179th generation. Twelve days after this a slight temperature disturbance was noticed, showing itself as a high morning exacerbation for three days. Bleeding for serum was undertaken on the 13th and 18th days.

Symptoms.—On the 22nd February, 1916, in the morning this horse was found pressing forwards into the wall, apparently unconscious of its surroundings. It was placed in a loose-box, where it now lay down. 9.30 a.m.: A marked inspiratory dyspnoea was present; the animal was still lying spread out on its side, shivering, distressed, but not struggling. 12.30 p.m.: The animal was found dead in the box.

Post-mortem report.—The condition was fair. Rigor mortis was present. Integument showed abrasions above the eyes. The mucous membranes of the natural openings were somewhat pale. The flesh was somewhat dark in colour. The subcutaneous tissue showed a markedly yellow tinge. The costal pleura was yellow; no abnormal contents in the pleural sacs. Diaphragm convexly forwards. Peritoneal cavity showed no abnormal contents. Lymph glands: the mandibulars were somewhat moist; the retropharyngeals appeared normal. The thyroids were somewhat pale. Tongue and oesophagus showed no abnormalities. Few gastrophilus larvae were found in the pharynx. The mucosa of the larynx and trachea was slightly pale yellow. The left lung was collapsed, on section slightly hyperaemic, of normal consistence. In the right lung were found some fibrous nodules, size of a pea. The pericardium contained 50 c.c. yellow fluid. Circulatory organs: both ventricles were empty. Right endocardium showed a few ecchymoses, the valves showed gelatinous infiltration at the base, left endocardium showed marked ecchymoses. Myocardium was pale and soft. The fat in the grooves of the coronary arteries was deep yellow. The liver had a light brown colour. The capsule had a pitted surface, the pits standing closely together; they were darker in colour than the surrounding raised network. On section, dark grooves and pits were seen, situated between a grey meshwork, in which occasionally the sunken septum could be made out. It was thus clear that the centre of the lobule was sunken whilst the periphery was raised. In many cases the central vein could be recognized as a fine pit in the depressed portion. The consistence was fairly firm. The spleen measured 42×21 cm. The pulp was dark and firm, the trabeculae were distinct. The supra renal glands showed no abnormalities. Kidneys: the capsule was easily detached; on section the parenchyma was yellowish brown. Stomach: a few gastrophilus larvae were present. Small intestines were normal, also the large intestines. Bladder contained turbid yellow urine. Nervous system: the brain showed no abnormalities. Bone marrow of humerus and femur showed haemorrhagic foci.

Pathological anatomical diagnosis.—Icterus. Slight hyperaemia of lungs; ecchymoses of both endocardiums, stasis, and parenchymatous degeneration of the liver.

Microscopical examination.—The lobules were by no means distinct, but their position could be made out by the position of the central veins and the connective tissue of the interlobular septa. All the blood vessels were gorged with red corpuscles. The zone surrounding the central vein, in contradistinction to the darker outer zone was light in colour. Only around the central vein were some liver cells or remnants of such, containing vacuoles; the nuclei were poor in chromatine. Round cells, smaller and larger ones, pigment, and red corpuscles were frequent, and a marked homogeneous substance of a reddish tinge filled the spaces between what remained of liver cells. The pigment was brown, granular, dust-like, and placed around or near to a nucleus (endothelial cells). Part of the pigment gave the Berlin blue and Turnbull's reaction. Sometimes in a cluster of pigment one part gave the reaction, whilst the remainder did not. The homogeneous reddish substance contained at certain distances round and oval shaped nuclei, representing probably the endothelial cells of the capillaries. In the periphery were situated more or less intact liver cells, the nearer the centre the more they were vacuolated. In one of the liver cells in the periphery a dyaster was noticed. In the septum were crowded numerous round cells with dark nuclei: they generally surrounded the bile-ducts and blood-vessels. In some parts they entered from the periphery of the lobules between the liver cells. Where the liver cells could be recognized they were not grouped in rows, but seemed to be out of their regular order. In the periphery surrounded by large cells was seen the lumen of bile-ducts.

Diagnosis.—Fatty degeneration and necrosis of the liver cells. Regeneration of bile-ducts. Cirrhosis. Emigration of round cells. Pigment. Stasis.

Case 8. Horse 10006. An aged brown gelding, obtained from the Defence Force on the 9th December, 1915.

Anamnesis.—It passed the mallein test on the 14th December, 1915. On the 18th December, 1915, it was submitted to immunization against Horse-sickness, receiving intrajugularly 5 c.c. virus of horse 9677, Tzaneen, 13th

generation; on the 24th intrajugularly 50 c.c. serum "O" 1711 and 5 c.c. virus of horse 9302 "O" 175th generation; on the 27th 300 c.c. serum "O" 1711. It passed through a very slight reaction from the eight to the fourteenth day, with only one high temperature exacerbation to 103.4° F. It was hyper-immunized on the 28th January, 1916, receiving 10,000 c.c. virus "O" 179th generation. On test the serum was found to be haemolytic; the horse was rejected and sent to the farm.

Symptoms.—27th February, 1916.—According to the report of the farm foreman this animal, used as a riding horse during the day, was seen at 6 p.m. standing in the camp, not feeding. 28th February, 1916.—At 6 a.m. the horse was found down, lying spread out, showing numerous scratches and wounds above the head and eyes, shoulder, and body. The poles in one corner of the camp showed blood marks where, during the night, the horse had apparently been pushing the head into the fence. The horse died about 8 a.m.

Post-mortem report.—The condition was fair. Rigor mortis was present. The visible mucous membranes were pale. The flesh had a normal colour, the subcutaneous tissue was slightly yellowish. Pleural cavities showed no abnormal contents. The diaphragm was convexly forwards. Peritoneal cavity was normal, peritoneum smooth and glistening. Mandibular lymph glands were moist and slightly hyperaemic, also the retro-pharyngeals. Thyroids were pale, the right showed a small strumia nodosa. Bronchial and mediastinal lymph glands were slightly hyperaemic. The tongue, oesophagus, and pharynx were normal. The larynx and cervical trachea contained some ingesta. The lungs were collapsed; on section they showed a normal appearance; there was some food in the main bronchi. Under the pleura of the right lung were some calcareous nodules. The pericardium contained 50 c.c. yellow fluid. Circulatory organs: both ventricles were empty. The epicardium showed a few ecchymoses. The right endocardium was yellowish brown; a few petechiae were present. The left endocardium showed marked extravasations; the valves were somewhat gelatinous. The myocardium was pale and somewhat softened. The pulmonary vessels showed no abnormalities, neither did the thoracic and the abdominal aorta. There was an aneurism in the branches of the anterior mesenteric artery. The liver showed fibrous filaments on the capsule, which was slightly shrivelled. The organ was somewhat small, showed sharp margins, and on section it was greyish in colour; a slightly mottled appearance was present; the consistence was fairly firm. The surface was pitted, the pits were of dark colour. On observing under a hand lens (6x) a distinct greyish network was noted, with thin slightly raised meshes, and in parts in the meshes sunken dark areas, which were in some places confluent. In the pit the small lumen of the central vein could be seen, sometimes cut crossways, sometimes longitudinally. One had occasionally the impression that the walls of the central veins were thickened. In the white ridges were seen the interlobular septa. Spleen measured 34 × 18 cm. On section pulp was of normal consistence, dark in colour. Stomach: fundus showed a few gastrophilus larvae, the mucosa was normal. The small intestines showed a slight hyperaemia. The large intestines showed slight hyperaemia. Bladder contained yellowish turbid urine, the mucosa was normal. The sexual organs were normal. The brain showed no abnormalities. Bone marrow of humerus was fatty; that of the femur showed marked haemorrhagic foci.

Pathological anatomical diagnosis.—Icterus, ingesta in trachea and bronchi, petechiae of mucosa of trachea, stasis, atrophy and parenchymatous degeneration of liver; slight hyperaemia of intestines.

Microscopical examination.—Liver: the section had somewhat a mottled appearance due to the presence of islands of yet normal liver cells surrounded by tissue devoid of them. Liver cells found in the periphery of the lobules bordering the interlobular septa were relatively intact. The septum contained numerous round cells. The liver cells contained numerous smaller and larger vacuoles. In the area surrounding the central vein only remnants of liver cells were recognized; the nuclei were surrounded by vacuolated plasma and numerous round cells. Light brown pigment was somewhat richly present, forming isolated granules and clusters; it was frequently found within the cell plasma and surrounding the nucleus. Practically all the pigment gave the Berlin blue and Turnbull reaction. Sudan stain showed rather large sized globules of fat in the peripheral and central cells of the lobules. Heart: the larger vessels and capillaries were rich in blood corpuscles. The Sudan stained sections showed most extensive fatty degeneration of the muscular fibres, some of which in their whole length were dusted with small and large sized globules,

so that the striation could no longer be recognized, and the nuclei were obscured. Kidney: large and smaller vessels and capillaries were distended and crowded with red corpuscles. Sudan stain showed extensive globular fat in practically all the tubuli contorti and tubuli recti.

Diagnosis.—Necrosis and fatty degeneration of liver cells, Stasis. Pigment. Fatty degeneration of the muscle of the heart. Fatty degeneration of the kidney.

Case 9. Horse 9812. An aged chestnut gelding, arrived on the 27th November, 1915, from the Defence Force.

Anamnesis.—It was submitted to the mallein test on the 30th November, 1915, and passed as healthy. From the 24th January, 1916, to the 28th February, 1916, this horse was submitted to a weekly dipping of seven-days' strength (Arsenite of soda $2\frac{1}{2}$ lb., soft soap 2 lb., and paraffin 1 gallon per 100 gallons of water).

Symptoms.—On the 1st March, 1916, the horse was brought up from the farm, the stockman reporting it as having gone blind. During the night it had escaped from the camp in which it was confined with a number of horses submitted to the same experiment. It was severely cut all over the body by the barbed-wire of the fence. Its condition was fair. When walking the animal pushed forwards into objects as if unable to see; the mouth was partially open, twitching of the lips was present. The horse was in a semi-comatose state. 12 noon: The horse walked round the box very slowly; it stood still when it had found support for its head, leaning against the wall or the manger. The lids of both eyes were swollen and the skin above the eyes was covered with whitewash from the wall; whitewash marks were also present on neck and on the side of the body. The horse was also observed to kick at the abdomen and look at intervals at its sides. The mouth was kept open, spasms of the lips were present, unphysiological positions of the limbs were noticed, viz., front limbs crossed, lifting of one of the hind limbs, resting head on the manger, raising head and neck, and rubbing nose against the wall up and down. The horse had not been noticed to feed; it had a tucked-up appearance. Abdominal respiration was present, the pulse was frequent, small, and soft. The extremities were cold. 2 p.m.: The horse was found standing quietly, with head right up against the wall, being apparently unconscious. 7.30 p.m.: Animal was found dead in box.

Post-mortem report.—The condition was fair. The integument showed numerous scratches on hips and side of body and abrasions above the eyes. The visible mucous membranes were pale. The flesh was somewhat pale. The subcutaneous tissue was markedly yellow. The costal pleura was yellowish. The diaphragm convexly forwards. Peritoneum was smooth and glistening and somewhat yellow. Lymph glands: Mandibulars and retro-pharyngeals were slightly oedematous. The thyroids were small and pale. The bronchial and mediastinal lymph glands contained small haemorrhages. The tongue, pharynx and oesophagus were normal. The larynx was slightly hyperaemic, cervical trachea was normal. The left lung was partially collapsed, numerous petechiae were present on the pleura; on section, a marked hyperaemia was found. There was a stasis in the right lung, the consistence was normal. The mucosa of the thoracic trachea was slightly yellow. The pericardium contained 120 c.c. of a reddish yellow fluid. Circulatory organs: the right ventricle was empty; the left contained partially coagulated blood. The epicardium showed numerous petechiae. The right endocardium was normal. The left showed a few ecchymoses. The myocardium was pale and somewhat soft. In the branches of the anterior mesenteric artery was an aneurism. The liver capsule showed fibrous filaments. Post-mortem changes were evident. The colour was somewhat dark brown. The capsule was shrivelled in parts and smooth in others. On section, some lobulation could be made out: a greyish lacerated margin forming a narrow slightly raised ridge mapping out some sort of meshwork, in the meshes of which were the slightly sunken lobules ($6\times$ magnified). In other parts no distinct picture could be made out, the surface being uniformly smooth. The spleen measured 39×18 cm., pulp on section was normal. Supra-renal glands were normal. Kidneys: Capsule easily detached, parenchyma on section was pale, the consistence was normal. Stomach: a few gastrophilus larvae were present. Slight hyperaemia in the fundus. Small and large intestines: mucosa slate coloured. Bladder contained some yellow urine. Sexual organs showed no abnormalities. Nervous system: Brain normal. Bone marrow showed normal fatty condition.

Pathological anatomical diagnosis.—Icterus. Wounds on head; hyperaemia of lungs and petechiae of pleura; hydropericardium. Petechiae on epicardium. Ecchymoses of left endocardium. Stasis and parenchymatous degeneration of the liver. Gastro enteritis catarrhalis.

Microscopical examination.—The normal lobular picture had disappeared. The situation of the lobules could, however, be made out. Their outlines were still present. In the septa was an accumulation of round cells, both of lymphocytic and polyblastic type, in no way abundant, but just sufficient to mark the periphery. There was no increase of any fibrillar connective tissue. Along the septa of some lobules liver cells were found with vacuoles. More frequent were pale-coloured cells containing a number of nuclei. The cells were concentrically arranged around a lumen and represented apparently a budding bile-duct. A number of them occasionally were found crowded together. Their cytoplasm was finely areolated, the outlines of individual cells were not visible, although a bile-duct as such could be recognized. Between the periphery and the centre of the lobule no intact liver cells could be recognized; some outlines of such enclosed a big vacuole, but they were very rare. Only an indefinite meshwork was left with the endothelial cells and connective tissue cells distinctly standing out and between them a homogeneous, or areolated and vacuolated substance. Here no liver cell nucleus could be recognized with certainty. The capillaries occupied the main portion; they were distended, and contained closely packed red corpuscles, between which the white corpuscles and the stained endothelial nuclei of the capillaries were conspicuous. Practically all endothelial cells contained pigment of a fine dust-like brown character. This pigment gave Berlin blue or Turnbull's reaction only in traces. Sudan stained sections showed particularly around the central veins large fat droplets; such droplets were also found through the lobules.

Diagnosis.—Necrosis and fatty degeneration of liver cells. Regeneration of bile-ducts. Stasis; pigment. Round cell infiltration of the septa.

Case 10. Horse 9989. An aged bay gelding obtained from the Defence Force on the 9th December, 1915.

Anamnesis.—Submitted on the 14th December, 1915, to the mallein test and passed as healthy. On the 18th December, 1915, it was put into an immunization experiment for Horse-sickness and received intrajugularly 5 c.c. virus of horse 8951, Tzaeneen 13th generation; on the 24th 50 c.c. serum "O," 1712 and 5 c.c. virus of horse 9302 "O," 175th generation; and on the 27th 350 c.c. serum "O," 1712. It developed a typical Horse-sickness reaction lasting from the seventh to the fourteenth day, succeeded immediately by a second reaction lasting to the twentieth day. The morning temperature during this period was never above 100° F., whilst the evening temperature only on one occasion reached 104° F.; on the average it was 102° F. The horse was submitted to hyperimmunization on the 31st January, receiving 10,000 c.c. virus of horse 9923 of 179th generation of the ordinary virus (O.). Subsequent to the infusion on the fourth and fifth day a rise of temperature was noticed for one day only, and reaching 105.6° F. on the morning of the fifth day, dropping to normal next morning. The pulse was 76 and 52 respectively on the corresponding days. The serum of this horse proved to be haemolytic, therefore the horse was discarded and sent to the farm Doornpoort. At Doornpoort on the evening of the 5th March, 1916, it was noticed to be ill. It died on the morning of the 6th and was brought in to the laboratory for post-mortem.

Symptoms.—When taken ill the horse showed the following symptoms: uneasiness and kicking against the abdomen. It sweated and was very excited, running and staggering about, always pushing forwards. It had finally to be tied up.

Post-mortem report.—The condition was fair. Rigor mortis was present. The conjunctiva was yellowish. Subcutaneous tissue showed in parts some gelatinous infiltration—it had a yellowish colour. The flesh was pale and somewhat moist. The pleural cavities contained no liquid. The diaphragm was convexly forwards; fibrous filaments were present on the peritoneal side of it. Peritoneal cavity contained no liquid. The bronchial and mediastinal lymph glands were hyperaemic and oedematous. Tongue, pharynx, and oesophagus showed nothing abnormal. In the larynx and cervical trachea was yellowish froth. Left lung was partially collapsed. Fibrous filaments were on the pleura as well as some extravasations and petechiae. On section of the lung several nodules with a haemorrhagic zone and a fibrous capsule

were noticed enclosing pus. The tissue was oedematous. The bronchial mucosa was slightly injected. Pleura of right lung showed some fibrous filaments; also some extravasations were present. On section of the lung a slight oedema was found. Mucosa of thoracic trachea was injected and slightly yellow. In the pericardium 20 c.c. straw-coloured liquid. Badly coagulated blood was present in the ventricles. On the epicardium were seen numerous petechiae and yellowish streaks. On the right endocardium were some ecchymoses and small slightly yellowish tinged fibrous patches. The left endocardium showed ecchymoses and yellow staining. The myocardium was pale and somewhat friable. The intima of the thoracic aorta was slightly yellow stained. Fibrous filaments were on the capsule of the liver, and underneath a calcareous nodule of the size of a marble. The colour of the parenchyma was somewhat bluish. The capsule was slightly shrivelled in parts and of a fine mottled appearance, somewhat pitted, the pits being lighter in colour. On section, a meshwork could be recognized, the meshes being slightly raised. The consistence of the liver was fairly firm. The spleen showed numerous petechiae in the capsule, the pulp was moist and soft, trabeculae were fairly distinct. Kidneys: the fibrous capsule was easily detached, the cortex was slightly yellow; the intermediary zone was slightly congested. The stomach contained some gastrophilus larvae; in the fundus was a patchy hyperaemia present. Small intestines showed a slight patchy hyperaemia. The caecum showed nothing abnormal; the colon showed patchy hyperaemia and a number of petechiae. The rectum showed a marked patchy hyperaemia and yellow staining. The mesentery was yellowish stained. The bladder contained clear yellow urine, and the mucosa had a few marked haemorrhages. Sexual organs showed no abnormalities. Nervous system showed nothing unusual. Bone marrow of the femur was fatty, in parts haemorrhagic.

Pathological anatomical diagnosis.—Icterus; slight oedema of lungs; parasitic nodules, fibrous pleuritis, ecchymoses on pleura, petechiae on epicardium and ecchymoses on the endocardium; cloudy degeneration of myocardium, marked parenchymatous degeneration of the liver; slight gastritis; colitis, haemorrhages in bladder.

Microscopical examination.—Examination with a hand lens (6x) of the liver fixed in formaline showed on section conspicuously a brownish meshwork apparently corresponding to the periphery of the lobules. The meshes were oval-shaped or elongated, sometimes semi-circular, and occasionally also branched. Within the mesh was a greyish, opaque substance in which a small lumen indicated the central vein. In the lobules cut longitudinally these were seen as grooves. Higher magnification: the wall of the central vein appeared slightly thickened; it had a hyaline appearance, staining well with eosine; elongated spindle-shaped nuclei of the endothelial cells were recognized lining the lumen of the veins. Surrounding these vessels were remnants of what appeared to have been liver cells at one time: they were vacuolated and the vacuoles were of different size, sometimes several in one cell and surrounding the nucleus. There was pigment present (and a good deal of deposit), occasionally collected in large clusters, but usually in small ones. Surrounded by this vacuolated zone was a more compact zone, in which there were practically no liver cells, at least none to be recognized with certainty. All the capillaries contained red corpuscles. Radiating from the septum were large elongated multi-nuclear cells with pale vesicular large nuclei mainly arranged in the periphery (budding bile-ducts). The septum itself contained an increased number of round cells of lymphatic and polyblastic type as well as fibroblasts.

Diagnosis.—Stasis; fatty degeneration and necrosis of the liver cells. Regeneration of bile-ducts; pigment; round cell infiltration of the interlobular septa.

Case 11. Horse 9949. An aged grey gelding, received from the Defence Force on the 9th December, 1915.

Anamnesis.—On the 14th December, 1915, it was malleined and passed as free from disease. On the 18th December, 1915, it was submitted to the immunization against Horse-sickness, receiving intrajugularly 5 c.c. virus of horse 9438, Tzaneen, 13th generation; on the 24th 50 c.c. serum "O." 1711 and 5 c.c. virus of horse 9302, "O." 175th generation; and on the 27th intrajugularly 380 c.c. serum "O." 1711. The horse showed a typical reaction with remittent fever exacerbations, lasting from the seventh to the eighteenth day. The pulsations during this period varied from 36 to 48. The maximum temperature on four days, the eleventh to the fifteenth day, was 104° F. The morning temperature during this period averaged 100° F. On the seventeenth

day the symptoms of a very slight Dikkop were noticed, the fossae temporales were level. All symptoms had disappeared on the twenty-first day. The horse was discharged and sent to the farm.

Symptoms.—On the 18th March, 1916, 9 a.m., the animal was brought back from the farm; it was restless and staggered from side to side. When placed in a loose-box it was staggering about, knocking the head against the wall. 2.30 p.m.: The animal was down, assuming the sternal position, and somewhat quieter. 19th March, 1916.—7 a.m.: The animal was found dead in the box. The walls showed blood patches extending from the ground to a height of about 5½ feet, all round the box.

Post-mortem report.—The condition was poor. The integument showed wounds and bruises on the head, particularly above the eyes and on lower part of the face. The visible mucous membranes of the eye showed haemorrhagic blotches. The subcutaneous tissue of the head showed blood suffusions. Pleural cavities showed no abnormal contents. Diaphragm was convexly forwards. Lymph glands: Mandibulars, retro-pharyngeals, bronchials, and mediastinals were normal. The thyroids appeared pale on section. Tongue and oesophagus were normal. Larynx and cervical trachea were normal. The lungs were partially collapsed; on section parenchyma was dark red, calcareous nodules were frequent. Some petechiae were found in the mucosa of the bronchi. The pericardium appeared normal. The right endocardium was slightly yellowish, the left showed ecchymoses. The myocardium was somewhat soft. The intima of the pulmonary vessels was slightly yellowish. The liver was small in size; on section, yellowish. A brown meshwork could be recognized on section; consistence fairly firm, calcareous nodules were frequent. Capsule was somewhat shrivelled, in parts unevenly corrugated. The spleen measured 39 × 16 cm.; the pulp was rather firm, the trabeculae were distinct. Supra-renal glands were somewhat pale. The kidney capsule was easily detached. Left kidney on section was pale, right kidney showed stasis. Stomach: mucosa of fundus was pale, few gastrophilus larvae were present. Small intestines showed in patches a slate-coloured mucosa, also the large intestines. Bladder contained some turbid urine. The brain was normal. Bone marrow was gelatinous.

Pathological anatomical diagnosis.—Icterus; oedema and hyperaemia of lungs; ecchymoses of left endocardium; atrophy, pigmentation and parenchymatous degeneration of the liver, atrophy of spleen; parasitic metastases in lungs and liver; fresh traumatism on head.

Microscopical examination of the Liver.—Under the low power (6×) the section showed the lobules with a greyish centre, in it the central vein as a small pit. It was surrounded by a darker zone. The interlobular septa appeared white and enlarged, irregularly outlined. The centres of the lobules contained no more liver cells, or only fragments of such. Their place was taken up by a not organized substance, within which were seen a few liver cells or fragments of liver cells containing vacuoles. There was much pigment present and fat in some parts of the section, none in others. The pigment was light yellowish brown; it gave the Berlin blue reaction in parts. There was a considerable increase of round cells in the septum, both lymphatic and polyblastic cells. In some parts the peripheral capillaries of the lobules were filled with red corpuscles.

Diagnosis.—Fatty degeneration and necrosis of liver cells, emigration of round cells into the septa, pigmentation.

Case 12. Horse 9938. An aged chestnut gelding, obtained from the Defence Force on the 9th December, 1915.

Anamnesis.—The horse was malleined on the 14th December, 1915, with negative result and passed as healthy. On the 18th December, 1915, it was immunized against Horse-sickness, obtaining intrajugularly 5 c.c. virus of horse 8951, Tzaneen, 13th generation; on the 24th 50 c.c. serum "O." 1711 and 5 c.c. virus of horse 9302, "O." 175th generation; and on the 27th 535 c.c. serum "O." 1711. It passed through a fairly mild Horse-sickness reaction with temperature exacerbations on the eleventh and twelfth day, reaching 104° F. on the fourteenth day, after which day the temperature returned to normal. The symptoms of Dikkop appeared and remained to the twentieth day, but they were never strongly developed. On the 21st January, 1916, the horse was submitted to the hyperimmunization, receiving 5000 c.c. virus of horse 9904 "O." 179th generation and 5000 c.c. of horse 9903, "O." 179th generation. On the eighth day after infusion the temperature rose and reached

105° F. on the ninth day; normal conditions were reached again on the twelfth day. The mucous membranes of the eyes examined on the tenth day were orange tinted; the pulse increased to 80 per minute, and in the blood the parasites, *Nuttallia equi*, were found in rare numbers; they were present on two successive days. The horse was treated with trypan blue and recovered. It was utilized for serum. On the 12th March, 1916, it was hyperimmunized for the second time to the amount of 10,000 c.c. of virus of horse 10057 of the ordinary virus, of the 180th generation. Subsequent to this hyperimmunization it showed a high temperature for three days.

Symptoms.—On the 21st March, 1916, the horse showed symptoms of colic, being restless, lying down frequently, and not feeding. 22nd March, 1916.—This morning the horse appeared somewhat dull and somnolent, not feeding, standing with head leaning forwards against the side of the stable; fresh bruises and wounds were noted above the eyes. 23rd March, 1916.—The horse died during the night.

Post-mortem report.—The condition was fair. Rigor mortis was present. The integument showed loss of hair and abrasions round both eyes and on left side of the frontal region. Some ingesta was found in the nostrils. The visible mucous membranes were slightly yellow. The flesh appeared rather pale. The subcutaneous tissue was generally yellow. Pleural cavities contained a little liquid. There were fibrous filaments on the costal pleura. Diaphragm was convexly forwards, fibrous filaments were present on both surfaces. Peritoneal cavity showed no abnormalities. Bronchial and mediastinal lymph glands were slightly enlarged and hyperaemic. Tongue on section was very yellow; mucosa of oesophagus likewise; some hyperaemia was noted in the pharynx. Mucosa of larynx and cervical trachea was slightly hyperaemic. Left lung was slightly collapsed, fibrous filaments and some yellow gelatinous infiltration of the pleura of the apical lobe; part of the main lobe was livid coloured. Parenchyma on section was pale and oedematous; some oedema and emphysema and some greenish consolidated foci were found in the apex. Ventral half of main lobe was consolidated and contained caverns filled with yellowish green substance; bronchi in these parts contained a greenish mucous substance. The right lung showed the pleura livid coloured; on section it was hyperaemic and oedematous. Near the ventral border of the main lobe were two small areas with commencing consolidations similar to that in the left lung. Thoracic trachea contained froth and greenish mucus. The mucosa was greenish stained. The pleura of the pericardium was hyperaemic and slight yellowish stained, an extensive deposit of fibrin was present. The pericardium contained 100 c.c. red-stained liquid. Both ventricles contained badly coagulated blood. The epicardium showed petechiae suggillations; it had a yellowish tinge. The myocardium was pale and soft. Intima of thoracic aorta was yellow. Liver: fibrous filaments were present on the capsule. The liver was somewhat flat with sharp edges. Some post-mortem changes were present. On section, a bright yellow colour of the parenchyma was striking, it was soft and friable, the lobules were indistinct. Spleen measured 53 × 27 cm., petechiae were in the capsule, the pulp was somewhat pale and moist, the trabeculae were distinct. The suprarenal glands appeared very yellow. Kidney capsule was easily detachable; on section, the parenchyma appeared yellow; it was also soft. Mucosa of fundus was slightly yellowish, gastrophilus larvae were rare. Small intestines: in parts the mucosa showed some ecchymoses, otherwise it was slate coloured. Large intestines: a patchy hyperaemia was present. A fair amount of fat was present in the mesentery. Some of the lymph glands were haemorrhagic. The bladder contained some greenish urine containing flocculi, the mucosa was deep yellow. The sexual organs showed no abnormalities. Bone marrow of femur and humerus showed haemorrhagic foci. The brain was of normal consistence and colour.

Pathological anatomical diagnosis.—Traumatism of head, general icterus; slight hydropericardium; extravasations and petechiae of epicardium and left endocardium; oedema and pneumonia gangrenosa. Atrophy and parenchymatous degeneration of the liver; hyperaemia of large intestines.

Microscopical examination of the liver.—Small power (6×). The lobules were not well defined. Around the central vein was seen a pigmented area which was partially surrounded by a whitish zone. Both areas formed on section two interlaced networks. In the outer whitish zone the connective tissue system of the septa could be recognized. In the periphery of the lobule were liver cells. These cells were, however, vacuolated; some were very large and contained a number of nuclei. They were budding bile-ducts undergoing

fatty degeneration. Some consisted almost entirely of one vacuole. Most of the liver cells appeared out of place and were mis-shaped. Between the centre and the periphery was situated a zone in which only a few liver cells could be recognized. Here a homogeneous substance occupied the place. Many cells showed only outlines of a cellular body without any structure. The structureless substance replaced the former liver tissue. In this substance a yellowish pigment was deposited. It gave the Berlin blue reaction only to a small extent. Where cellular bodies could be distinguished the pigment was often found within them. The Sudan stain showed the presence of many large-sized fat globules in the broken-down liver cells.

Diagnosis.—Necrosis of liver cells; fatty degeneration and pigment; regeneration of bile-ducts.

Case 13. Horse 9632. An aged grey gelding, obtained from the Defence Force on the 28th August, 1915.

Anamnesis.—The horse passed the mallein test on the 2nd September, 1915. On the 12th December, 1915, it was passed into the experimental stable and kept under observation. On the 4th January, 1916, it was put in a horse-sickness experiment, receiving intrajugularly 5 c.c. virus of horse 9274, "O.P." 1st generation; and on the 8th January, 1916, also intrajugularly, 280 c.c. serum "O" 1711 and 5 c.c. virus of horse 9302 "O" 175th generation. It developed rather a severe reaction, lasting from the eighth to the eighteenth day, with a fever exacerbation to 107° F. on the sixteenth and seventeenth days. A slight Dikkop was noticed on the twenty-third day, but disappeared on the twenty-sixth day. The horse was hyperimmunized on the 2nd February, 1916, with 2500 c.c. virus of horse 9923 and 2500 c.c. of horse 10066 "O" of the 179th generation. There were some irregular fever reactions after this. The horse was sent to the farm at Doornpoort on the 1st March, 1916.

Symptoms.—The farm foreman reported that this animal on the evening of the 28th March, 1916, showed symptoms of staggers and died on the farm on the 29th March, 1916. It was brought to the laboratory for post-mortem.

Post-mortem report.—The condition was poor. Rigor mortis was passing off. The integument showed abrasions on the hips, supra orbital processes, and zygomatic arches. The visible mucous membranes were slightly yellow. The flesh somewhat pale. The subcutaneous tissue was yellowish. The costal pleura was yellowish, no liquid in the sacs. The diaphragm convexly forwards. Mandibular lymph glands were slightly hyperaemic. The retro-pharyngeals markedly hyperaemic. Bronchial and mediastinals also hyperaemic. The thyroids were pale. The tongue on section appeared yellow, oesophagus and pharynx appeared slightly yellowish. Larynx and cervical trachea contained a reddish foam. Both lungs were collapsed; on section, marked stasis was present and the parenchyma was somewhat moist; blood-stained foam was contained in the bronchi. The thoracic trachea contained reddish yellowish foam; the vessels of the mucosa were injected. The pericardium contained 250 c.c. of a reddish yellow liquid. Circulatory organs: blood and plasma coagula were present in both ventricles. The vessels of the epicardium were injected. The right endocardium was brownish yellow, the left one showed marked ecchymoses, the valves were reddish brown stained. Intima of thoracic and abdominal aorta somewhat yellow. Liver: a few fibrous filaments on capsule. Parenchyma was partially shrivelled and pitted, the pits being dark in colour; on section, had a mottled appearance, colour was from light yellow to dark red, in parts almost haemorrhagic. Consistence was slightly tough. Spleen measured 48 × 17 cm., pulp on section was dark in colour; trabeculae were distinct, the consistence was normal. Supra-renal glands were normal. The kidney capsule was easily detached; on section, the right kidney appeared dark in colour, the left pale and slightly yellow. The fundus was slightly hyperaemic, gastrophilus larvae were present. The mucosa of the small intestines was dark slate coloured. Large intestines: mucosa of all portions were slate coloured. Bladder contained a turbid yellow urine. Sexual organs showed no abnormalities. Nervous system: the brain was normal in consistence and colour. Bone marrow of femur and humerus showed haemorrhagic foci.

Pathological anatomical diagnosis.—Icterus; oedema and stasis of both lungs, hydropericardium, ecchymoses of left endocardium, fatty degeneration of myocardium, atrophy, pigmentation and parenchymatous degeneration of liver. Gastro-enteritis catarrhalis.

Microscopical examination.—With a low power (6x) around the central vein an area of dark appearance could be recognized; it was bordered by a

white zone. The septa did not appear enlarged. Fat was present throughout the whole lobule, in particular fairly frequent in the periphery, and corresponded to the white zone described above. With higher magnifications the outlines of the lobule could be traced by the septa. Within the lobule the structure was upset. There were no regular liver cell rows, practically there were no intact liver cells left, or only very few, and if present principally in the periphery. The greater part of the lobule was occupied by a structureless substance. Outlines of cells, consisting of a homogeneous substance, were sometimes recognizable. Few normal liver cell nuclei could with certainty be made out. They had disappeared or were very small and pycnotic. A differentiation from the connective tissue cells was not always possible; also the endothelial nuclei were small and pycnotic. Much pigment was present; it gave the Berlin blue reaction. In the interlobular septa was a slight increase of round cellular infiltration present. In the degenerated lobule white corpuscles of polynuclear and round cell type could be recognized.

Diagnosis.—Atrophy, necrosis, and fatty degeneration of liver cells; pigment; round cell infiltration of the septa.

Case 14. Horse 9880. A chestnut gelding, seven years old, obtained from the Defence Force on the 4th December, 1915.

Anamnesis.—Submitted to and passed the mallein test on the 7th December, 1915. From the 24th January, 1916, to the 24th April, 1916, this horse was submitted to a dipping experiment at a weekly interval. The dip consisted of arsenite 2½ lb. and aloes 5 lb. per 100 gallons of water, with a film of linseed-oil and paraffin on the surface.

Symptoms.—In the afternoon of the 1st April, 1916, the horse was noted ill, showing signs of Staggers. It was placed in a loose box, but no unusual attitude could be noted. Temperature, pulse, and respiration appeared normal. 2nd April, 1916.—9 a.m.: The horse was not paying much attention to its surroundings. Whitewash marks were present in the regions above the eyes, of the nose, of the shoulders, and knees. Slight fresh bruises and wounds in the skin of the supra orbital processes. The horse took up unphysiological positions with the legs, viz., front limbs crossed, hind limbs spread apart, one leg placed in front of the other. Head and neck were turned slightly to one side, or the head was pressed forwards against the wall. There were no violent symptoms present. The horse was somnolent and did not feed. 3rd April, 1916.—9 a.m.: The somnolence was more marked. The horse walked round in the box very slowly, with front limbs somewhat spread, head and neck lowered and slightly turned to one side, or it remained standing either with the head under the manger, or leaning it against the wall. Unphysiological position of the limbs was taken up. Flies were noticed to settle on the animal. The upper eyelids were very much swollen; above each eye there were fresh wounds covered with whitewash. Slight salivation, slight spasms of the lips were present. 3.30 p.m.: Twitchings of lips frequent and more marked. When led out the horse had an uncertain gait, especially marked in the front limbs. The salivation had increased. 4th April, 1916.—The swelling of the upper and lower eyelids decreased. The animal brightened up and was no longer so dull, also took more notice of its surroundings. It made attempts at feeding. The gait also showed improvements. 5th April, 1916.—The animal showed further improvement, feeding more regularly. 6th April, 1916.—Animal appeared normal in gait and feeding.

On the 5th July, 1916, this horse was immunized, receiving intrajugularly 5 c.c. virus of horse 10319 Tzaneen 13th generation; on the 12th 180 c.c. serum "O" 1717 and 5 c.c. virus of horse 10036 "O." 176th generation; and on the 15th, also intrajugularly, 265 c.c. serum "O." 1717. The horse showed a reaction from the sixth to the seventeenth day, and the symptoms of a very slight Dikkop from the fourteenth to the twenty-first day. It was discharged as recovered and sent to the farm.

Case 15. Horse 10150. A seven-year-old grey gelding, obtained from the Defence Force on the 3rd February, 1916.

Anamnesis.—On the 6th February, 1916, it was submitted to the mallein test with negative result. On the 8th February, 1916, it was put in an immunization experiment against Horse-sickness and received intrajugularly 5 c.c. virus of horse 9677 Tzaneen, 13th generation; on the 14th 50 c.c. serum "O." 1713 and 5 c.c. virus of horse 9302 "O." 175th generation; and on the 17th, also intrajugularly, 400 c.c. serum "O." 1713. It had a Horse-sickness reaction, which was complicated with biliary fever. It showed yellow mucous membranes.

on the 25th February, 1916. On microscopical examination *Nuttallia equi* was found. The horse was then treated with intrajugular injections of 100 c.c. of 1 per cent. solution of trypan blue, and 10 grammes arsenophenylglycine in 1000 c.c. physiological water, and on the 26th the trypan blue injection was repeated. There was a sudden drop of temperature to subnormal next morning, from which time the temperature continued normally and remained normal up to the 30th March, when the horse was discharged and sent to the farm.

Symptoms.—On the 10th April, 1916, it was noticed ill and sent back to the station. It was reported as having shown symptoms of Staggers, viz., running about wildly and pushing forwards until it was stopped by some object, against which it then pushed with head lowered, taking up unnatural positions of the limbs and being apparently unconscious. Some difficulty was noticed in staling, no urine was passed for some time; then on its way to the laboratory it was noticed to have staled nine times, only passing small quantities at a time. On examination the animal appeared slightly tucked-up; signs of perspiration in the region of the neck were present. The animal had a peculiar gait when led: it lifted its front limbs abnormally high, it swayed from side to side; peculiar twitchings of the lips and masticating movements with some foamy salivation were present. The animal received a dose of chloral hydrate and was placed in a loose box. At 2 p.m. the horse was noticed pressing forwards into the corner of the box with great force, closing up its nostrils so that breathing became difficult, the symptoms of an inspiratory dyspnoea being so produced. The tail was lifted, the horse straining to pass urine, some drops were running from the sheath. At 4 p.m. the animal was found in sternal position with head and neck turned to one side; the breathing was now normal. Fresh wounds above both eyes were present. The temperature in the morning was 100.8° F.; it rose to 104° F. that evening. The horse died during the night.

Post-mortem report.—The condition was poor. Rigor mortis was present. Integument showed decubitus on hips, shoulders, and traumatism in frontal region. The visible mucous membranes were somewhat pale. The flesh appeared somewhat opaque and dry. Subcutaneous tissue was slightly yellowish. In pleural cavities no abnormal contents. The diaphragm was convexly forwards. Bronchial and mediastinal lymph glands were slightly hyperaemic; retropharyngeals and mandibulars were moist. Tongue, pharynx, and oesophagus showed no abnormalities. Larynx and cervical trachea were normal. Left lung was partially collapsed; fibrous filaments on pleura of main lobe, parenchyma on section moist and slightly darker in colour. Right lung on section rich in blood, some foam in bronchus. Thoracic trachea was slightly hyperaemic and contained foam. The pericardium contained 250 c.c. of a yellow fluid. Circulatory organs: Coagulated blood was found in both ventricles. The epicardium showed petechiae, the base was gelatinous. Right ventricle was somewhat dilated and its endocardium was pale; the left endocardium showed marked ecchymoses. The myocardium was pale and opaque, slightly dry. The pulmonary vessels were normal. Thoracic aorta showed no abnormalities. Liver: fibrous filaments on capsule of oral surface. The capsule was otherwise smooth, in parts pitted, the pits fairly widely separated. On section much blood escaped. The lobules could be recognized by their yellow periphery, the central veins appeared filled. The consistence was somewhat firm. The margins were fairly sharp. Spleen measured 37 × 18 cm. On section the parenchyma was dark red, the trabeculae were distinct, consistence was normal. Kidneys: capsule was easily detached. The organ on section showed pale areas in the cortex. Consistence normal. The stomach was fairly well distended with dry ingesta; the fundus showed patchy hyperaemia, *gastrophilus* larvae were present. Small intestines: slate bluish discoloration and patches of hyperaemia. Large intestines: slight patchy hyperaemia in the colon. The rectum was normal. The omentum showed a slight yellowish tinge. The bladder contained clear yellow urine, the mucosa was hyperaemic, and ecchymoses were present. Sexual organs showed no abnormalities. Nervous system: the brain was of normal colour and consistence. The bone marrow of the humerus was fatty and slightly hyperaemic in patches; in the femur the hyperaemia was more marked.

Pathological anatomical diagnosis.—Slight icterus and anaemia. Hydro-pericardium, ecchymoses of left endocardium and petechiae of epicardium. Atrophy and parenchymatous degeneration of the liver; hyperaemia of stomach and intestines; hypostasis of right lung; hyperaemia of bladder.

Microscopical examination.—Low power (6×). The section had a black and white mottled appearance. The white lines formed a reticulum, in the meshes of which were black areas. The centre was occupied by a pit (*vena centralis*); in parts the meshwork was formed by the black portion and the white ones were enclosed. Higher magnification: the liver cells had disappeared in the greater part of the lobule, and in their place were red corpuscles, the relatively intact liver cells were mostly in the periphery. There were also some within the lobule irregularly shaped or traces of such liver cells. They contained small fat droplets. The connective tissue was not increased, and there were no budding bile-ducts. There was much pigment present in the parts in which the liver cells were destroyed. Only the smaller portion of the pigment gave the Berlin blue and Turnbolls reaction. Central veins and sublobular veins were engorged, and the centre of many lobules consisted of pure blood.

Diagnosis.—Fatty degeneration and necrosis of the liver cells. Stasis; pigment.

Case 16. Horse 9884. An aged bay gelding, received on the 4th December, 1915, from the Defence Force.

Anamnesis.—On the 7th December, 1915, passed the mallein test with negative results. From the 24th January, 1916, to the 10th April, 1916, it was submitted to a dipping experiment at seven days' interval. The dip consisted of arsenite of soda $2\frac{1}{2}$ lb., aloes 5 lb. per 100 gallons, with a film of linseed and paraffin on the surface. On the 11th April the horse was found dead. It had been missing since the previous night. The foreman reported that the animal must have leaped over the fence, and on examination of the trail it seemed as if the animal was staggering in its walk. The carcass was brought in the same morning, but was found to be too decomposed for post-mortem examination.

Epicrisis.—Although the animal was not seen sick during life, there can be no doubt that it died of acute Staggers. The fact that it had forced its way through the fence and died subsequently speaks for such a diagnosis.

Case 17. Horse 10109. An aged bay gelding, obtained from the Defence Force on the 3rd February, 1916.

Anamnesis.—On the 6th February, 1916, malleined and passed as healthy. On the 8th February, 1916, submitted to immunization against Horse-sickness, receiving intrajugularly 10 c.c. virus of horse 9438, Tzaneen 13th generation; on the 14th 50 c.c. serum "O." 1711 and 5 c.c. virus of horse 9302 "O." 175th generation; and on the 17th, also intrajugularly, 380 c.c. serum "O." 1711. The horse passed through a typical Horse-sickness reaction, the temperature being of a remittent character, reaching 105° F. in the evening and sinking below 101° F. in the morning. The reaction lasted from the ninth to the sixteenth day. Symptoms of Dikkop appeared on the seventeenth day, but were only mild, and passed off on the twentieth day. There was a complete recovery, and a normal course of temperature up to the 12th May, when a slight rise in the morning temperature to 101° F. was noticed, and again a rise to 103° F. on the 14th May in the evening. The horse was found dead in the stable next morning.

Post-mortem report.—The condition was under fair. Rigor mortis was complete. The anus was open; some discharge was present in the nostrils. The visible mucous membranes were pale. Subcutaneous tissue was yellowish. Blood was dark and stained well. The serum was haemoglobin coloured. The fat was bright to dark yellow. The costal pleura showed wine red discoloration along the blood-vessels. Diaphragm convexly forwards; fibrous filaments were seen on the peritoneal side. Peritoneal cavity showed some haemoglobin stained fluid. The peritoneal serosa was slightly yellowish. Lymph glands: mandibulars were slightly enlarged and moist and dark in colour; sub-parotids slightly yellowish; retro-pharyngeals somewhat dark in colour; upper and lower cervicals were oedematous; bronchials and mediastinals appeared normal. Thyroids were normal. Tongue slightly roughened; the pharynx and oesophagus appeared normal. Mucosa of cervical trachea was slightly yellow and contained some froth. Mucosa of larynx was reddened and foam was present. The dorsal side of the velum palatini was black; mucosa of radix linguae was greyish; the epiglottis was injected. The lungs were collapsed; some emphysema was present in the anterior lobes and basal border of main lobe. Left lung showed areas of superficial atelectasis. The lungs on section were dark in colour, foam was present in the bronchi, red staining of intima of pulmonary vessels. Thoracic trachea was full of froth. Intima of thoracic aorta was red stained. The pericardium contained 120 c.c. of dark-coloured liquid. Both ventricles

were empty. The epicardium was diffusely wine red, and a wine red imbibition was noticeable along the coronary artery. Left endocardium was slightly wine red. Some ecchymoses under the valves, on the septum, and in *musculi papillares*. Right endocardium was diffusely red. Intima of coronary artery was red. Myocardium was pale, opaque, and slightly soft. Liver appeared small; fibrous filaments on posterior face, the edges were sharp; capsule slightly shrivelled, the section appeared light yellowish-brown. The consistence was fairly hard, the lobules were not very distinct; pancreas was soft and moist. Spleen slightly enlarged, 55×27 cm. The pulp was dark in colour, the trabeculae and follicles were distinct, the consistence was fairly firm. Supra-renal glands: the cortex was pale, the medulla was red. The kidneys were poor in fat, the fibrous capsule was easily detached, on section wine red and consistence somewhat softened. Stomach: mucosa of fundus and pylorus were slate coloured, *gastrophilus* larvae were present. Small intestines: slate colour of mucosa. Large intestines: mucosa was slate coloured. Intima of abdominal aorta was wine red. Intima of cranial mesenteric artery was roughened and a thrombus was present. Mesenteric lymph glands were oedematous. The urinary bladder was full of black urine, the mucosa showed petechiae. Sexual organs showed no abnormalities. Brain was pale yellow. Bone marrow of humerus was yellow and a red patch was present in the cortex; the femur contained also a haemorrhagic focus.

Pathological anatomical diagnosis.—Dissolutio sanguinis; haemoglobinuria; icterus; anaemia; oedema pulmonum; oedema of the lymph glands; emphysema pulmonum of the apex; ecchymoses of left endocardium; aneurisma of mesenteric artery; tumour splenis; atrophy and parenchymatous degeneration of the liver.

Microscopical examination.—In one of the sections small holes were seen, caused by gas bubbles. The piece was greenish; no definite lobulation could be distinguished, although an indefinite marking was noticeable. The septa were enlarged, but not by new connective tissue cells; the fibres simply seemed to be soaked or infiltrated with liquid; everything seemed homogeneous. The liver cells were practically all gone, and in their place was a finely granular substance, taking a slight pinkish stain. There were some liver cells scattered about containing vacuoles. There were more in the periphery than elsewhere. Here also multinuclear cells were seen, some with vacuoles and pigment. Also the walls of the central veins seemed thickened and homogeneous. Brown yellow pigment was packed in liver cells, of which sometimes only the outlines could be seen. About half of the pigment gave the Berlin blue and Turnbulls reaction.

Diagnosis.—Acute necrosis of liver cells; pigment and fatty degeneration. Stasis. Regeneration of bile-ducts.

Case 18. Horse 10094. An aged light grey gelding obtained from the Defence Force on the 3rd February, 1916.

Anamnesis.—It passed on the 6th February, 1916, the mallein test and was admitted to an immunization experiment on the 8th February, 1916, receiving intrajugularly 5 c.c. virus of horse 8951 Tzaneen 13th generation; on the 14th 50 c.c. serum "O." 1713 and 5 c.c. virus of horse 9302, "O." 175th generation; and on the 17th, also intrajugularly, 445 c.c. serum "O." 1713. It showed a rather severe reaction, lasting from the ninth to the eighteenth day, with evening temperature above 104° F. and morning temperature of 102° F. It showed congested mucous membranes of the eyes on the fifteenth day. On the 19th February, 1916, it was hyperimmunized, receiving 5000 c.c. virus of horse 10195 and 5000 c.c. virus of horse 10193 "O." 180th generation. As a result of this infusion it developed Piroplasmosis on the seventh day, lasting to the thirteenth day, with a temperature of 105.8° F. on the eighth and ninth days. There was a slight orange discoloration of the conjunctiva with ecchymoses. The examination of blood revealed the presence of *Nuttallia equi* on three days. Subsequent to this reaction a further one developed on the 8th April, 1916, lasting to the 17th April, 1916, in which the mucous membranes of the eyes appeared ecchymosed. *Spirochaetes* were found in the blood, and on the 14th April, 1916, again *Nuttallia equi*. The horse received on two occasions after an interval of three days an intrajugular injection of 10 grammes of arsenophenyglycine, the last dose supplemented by an injection of 100 c.c. of 1 per cent. trypan blue. The horse recovered.

Symptoms.—Loss of condition was noticed from the 13th April, 1916; on the 20th it showed a stiff gait in the hind limbs; on the 22nd the gait was noticed to be wobbly, the hind limbs dragging. On the 24th the animal went

down; it rose in the afternoon and was now noticed leaning against the manger. There were abrasions noted in the skin above both eyes, and marks of white-wash were present. The upper lip was swollen. The mucous membranes of the eye showed ecchymoses. In moving about it could be noticed that the horse pushed slightly forwards. Flies were settling on the animal without being taken notice of. On the 25th April, 1916, in the morning the swelling of the nose, upper and lower lip had increased. The tongue was sticking out between the teeth. The front legs were kept in an unnatural position, the one forwards and the other backwards and spread apart. The head was kept hanging. The eyelids were swollen, the mucous membranes were congested. The animal kept the eyes closed; it was apparently unconscious of what was happening around it. At times the front legs suddenly slipped straight forwards so that the horse came into a sternal position with its body; in this position the animal made several attempts to rise. At 3 p.m. the same day the animal was found again standing on its legs pressing the head firmly forwards in the corner of the box, compressing the nose so that the breathing was interfered with and the noises of dyspnoea were heard. At 7 p.m. the animal was found again in the sternal position as described above, with front legs stretched out, and the neck and head inclined to one side. The animal was semi-comatose. Later in the evening it was found spread out on its side. It died during the night.

Post-mortem.—The condition was fair. Rigor mortis was present. The integument showed abrasions over both eyes and hips. The conjunctiva was ecchymosed. The flesh was somewhat pale. The subcutaneous tissue had a yellowish tinge. Pleural cavities contained about 1 litre of reddish fluid. The diaphragm was convexly forwards. Fibrous filaments were present on caudal surface. The peritoneal cavity contained some reddish liquid. Lymph glands: retro-pharyngeals were hyperaemic; mandibulars appeared normal; bronchials and mediastinals were hyperaemic. The tongue tissue on section was yellow. Pharynx slightly hyperaemic, oesophagus normal. Both lungs were partially collapsed; the pleura had a livid colour with a number of petechiae and patches of yellow gelatinous infiltration. On section the lungs were mottled, hyperaemic, and oedematous. Bronchi contained foam, mucosa was injected. Partially coagulated blood was present in both ventricles of the heart. The epicardium was pale yellowish, the right endocardium likewise, with some ecchymoses. Myocardium was pale and softened. Aorta was normal. A small aneurism was present in the cranial mesenteric artery. Liver: fibrous filaments and patches were on the capsule. The edges were fairly sharp. On section the tissue appeared bright yellow. Consistence was rather firm. Spleen showed some fibrous filaments and patches on capsule, the parenchyma was rather dry, trabeculae were very distinct. Supra-renal glands were normal. Kidneys: capsule was easily detached. On section pale yellowish, consistence somewhat soft and friable. Stomach: few *gastrophilus* larvae were present. The mucosa was pale. Small intestines: slate-coloured mucosa. Large intestines: slate-coloured mucosa. Rectum showed patchy hyperaemia. Bladder: some turbid yellow urine was present. Mucosa showed patchy hyperaemia. Brain showed a normal appearance. Bone marrow of femur and humerus was fatty and in parts haemorrhagic.

Pathological anatomical diagnosis.—Anaemia. Icterus; hydrothorax; hydropericardium, ascites; hyperaemia and oedema of lungs; fatty degeneration of myocardium and kidneys. Atrophy, pigmentation, and degeneration of the liver.

Microscopical examination.—The unstained liver section was green. The septa appeared thickened and bordered irregularly shaped lobules. An enormous quantity of yellow pigment was present, forming clusters of different size, corresponding somewhat in their outline to the shape of liver cells. Some of the clusters were confluent, forming a large mass of pigment. The greater part of this pigment gave the Berlin blue and Turnbulls reaction. Budding bile-ducts in the form of light-coloured multinuclear cells were present; some of the bile-ducts were differentiated. Sudan stain showed the presence of fat in large quantities, large and small drops. Fat and pigment were seen together in the same cells. In the outer zone of the lobule the liver cells appeared to be complete, not so in the centre. Here the cells were absent and a structureless substance occupied their place, in which a few blue stained nuclei were visible.

Diagnosis.—Necrosis and fatty degeneration of liver cells. Pigment. Regeneration of bile-ducts.

Case 19. Horse 9831. An aged chestnut gelding, obtained on the 22nd November, 1915, from the Defence Force.

Anamnesis.—It was malleined on the 30th November, 1915, with negative results. From the 24th January to 17th April, 1916, this horse was submitted to a weekly dipping in a solution of arsenite of soda $2\frac{1}{2}$ lb. and aloes 5 lb. per 100 gallons, with a linseed-oil and paraffin layer on the surface.

Symptoms.—On the 22nd April, 1916, the horse was reported ill with symptoms of staggers, pushing forward so violently that a number of natives were required to hold it back. It was brought with difficulty to the laboratory in a float, staggering whilst backing out of it. The mucous membranes of the eyes were yellow; some ecchymoses were present. After having been placed in the loose box the horse stood for a while somnolent, with head hanging; later it was down and the breathing was laboured and rapid. It died the same afternoon.

Post-mortem report.—The condition was fair. The integument showed fresh bruises on near eye and decubitus on hips. There were incisions of the lips caused by the incisor teeth. The conjunctiva was slightly yellowish. The costal pleura was slightly yellowish tinged. Diaphragm convexly forwards. Peritoneal cavity contained some yellowish fluid. Petechiae were present on the serosa of the colon. A yellow gelatinous infiltration was found in the region of the kidneys. Lymph glands: mandibulars were normal, bronchials and mediastinals were black pigmented. Tongue, pharynx, and oesophagus were normal. Mucosa of cervical trachea was slightly yellowish. Petechiae were present on the epiglottis. Right lung was collapsed; on section it showed hyperaemia and slight oedema; petechiae were on the mucosa of the bronchus. Left lung contained two caseous nodules. Mucosa of thoracic trachea was slightly yellow. The pericardium contained 50 c.c. saffron coloured liquid. The left endocardium was yellowish and ecchymosed, the right endocardium was yellow. The epicardium was ecchymosed at the base and along the sulci. The myocardium appeared normal. Intima of thoracic aorta was yellowish. Liver: the organ appeared somewhat small, on section yellowish, the consistence was fairly firm. The surface of the capsule was slightly pitted; on section a meshwork was noticeable in the parenchyma of grey meshes of irregular size and outlines, standing out as ridges, the spaces between the meshes were dark in colour and sunken. The greyish meshes contained the interlobular septa. Spleen: capsule was yellowish, pulp on section firm, trabeculae were distinct. Supra-renal glands normal. Kidney capsule was easily detached; the section appeared pale yellowish, the pelvis was gelatinous. Fundus showed a few superficial erosions. Small intestines: blood-stained contents were present; the mucosa was haemorrhagic in parts. No abnormalities were noticed in the large intestines. The bladder contained yellow urine; few petechiae were present on the mucosa. Sexual organs were normal. Nervous system: brain of normal appearance. Bone marrow of humerus was fatty, that of femur showed a haemorrhage.

Pathological anatomical diagnosis.—Icterus; hyperaemia and slight oedema of both lungs; petechiae of epiglottis and bronchi; ecchymoses of epicardium and left endocardium; atrophy and parenchymatous degeneration of the liver; erosions of stomach; haemorrhages in small intestines; petechiae of bladder; wounds on integument.

Microscopical examination of the Liver.—Lower power (6x). The section showed a reticulum of light coloured meshes, enclosing irregularly outlined dark coloured islands. The septa were in white meshes; the portal veins stood out as black points. The central veins were not distinct. Higher power: the central veins were filled with blood. The blood corpuscles were fused together. The outlines of the veins were not quite clear, merging into the central zone of the lobule, in which there were no more liver cells left. The nuclei present were of the connective tissue type; the liver cells present were vacuolated and showed fat globules. There were light coloured cells present adjoining the periphery of the lobule; they were the giant cells of the budding bile-ducts and surrounded by round cells of polyblastic type and by fibroblasts. These buds entered between the remaining liver cells. There was some light brown pigment present in the central portion, forming large clusters. About half of it gave the Berlin blue reaction. Also some of the newly formed bile-ducts showed fat granules.

Diagnosis.—Necrosis and fatty degeneration of liver cells. Regeneration of bile-ducts. Stasis. Pigment. Emigration of round cells.

Case 20. Horse 10159. A cream coloured gelding, 7 years old, obtained from the Defence Force on the 3rd February, 1916.

Anamnesis.—On the 6th February, 1916, it was submitted to the mallein test and passed in as healthy. On the 8th February, 1916, submitted to immunization against Horse-sickness, receiving intrajugularly 5 c.c. virus of horse 9765, Tzaneen, 14th generation; on the 14th 50 c.c. serum "O." 1712 and 5 c.c. virus of horse 9302, "O.", 175th generation; and on the 17th, also intrajugularly, 410 c.c. serum "O." 1712. It developed a mild reaction of Horse-sickness from the seventh to the seventeenth day. On two occasions, twelfth and thirteenth days, the temperature touched 103.6° F. in the evening. The morning temperature averaged 101.3° F. The horse was hyperimmunized on the 22nd March, 1916, with 2500 c.c. virus of horse 10188, "O.", 180th generation, and 2500 c.c. virus of horse 10187, 180th generation, and 5000 c.c. virus of horse 10187 on the 23rd. On the 30th March, 1916, it showed a rise in temperature, and on the following day its blood was examined and found to contain *Nuttallia equi* in rather frequent numbers. It was then injected with 100 c.c. of 1 per cent. trypan blue solution and 10 grammes arsenophenylglycine solution in 1000 c.c. physiological water. The parasites disappeared after this, and the temperature remained normal. The horse was bled on the 15th and 19th April for serum, the temperature at this time being normal.

Symptoms.—22nd April, 1916. The horse was reported to be suffering from Staggers. It was placed in a loose box and went down immediately. When lifted up it took up the position with the head leaning into the corner and slightly pushing forwards. Later it turned round in the opposite corner and urinated normally. The urine was clear. On the 23rd April, 1916, it was found down in a sternal position. It showed abrasions and slight swelling above the eyes; the nostrils and lips, were also slightly swollen and showed bruises. The mucous membranes of the eyes were distinctly yellowish. Flies were settling on the animal. It was semicomatose, bending its head and neck somewhat to the right. It died the same day.

Post-mortem report.—Autopsy was made shortly after death. The condition was fair. Rigor mortis was not present. The integument showed bruises and wounds on the skin of the supra orbital processes and zygomatic arches, and on several prominent parts of the body. Visible mucous membranes were pale yellowish. The tongue was hanging from the mouth. The subcutaneous tissue was yellowish. The flesh was somewhat brownish; also the blood had a brownish hue, it was not coagulated. The pleura was yellowish. Diaphragm was convexly forwards; fibrous filaments were present on both sides. The serosa of the peritoneal cavity was yellowish. Lymph glands: mandibulars and retro-pharyngeals on section were slightly yellow. Tongue tissue was yellowish. Pharynx and oesophagus appeared normal. Mucosa of larynx and cervical trachea was yellowish. The thyroids were pale and glistening on section. The right lung was partially collapsed. Fibrous patches were present on the pleura. On section parenchyma had a normal appearance. Some emphysema was present in the apices. Left lung showed some yellowish fibrous patches on the pleura of the body. The tissue was pale on section. A consolidated yellow focus was present under the dorsal border of the middle portion of the main lobe; yellowish exudate was present in the bronchus. Mucosa of thoracic trachea was yellowish. The pericardium contained 70 c.c. clear yellow liquid. The heart was flabby (not set). Both ventricles were almost empty. Left endocardium was yellowish and ecchymosed, right endocardium likewise; the valves were yellowish. The myocardium was opaque. Right pulmonary artery contained a white thrombus; intima of the vessels was yellowish. The intima of aorta was slightly yellowish. Liver was small and had sharp edges, consistence harder than normal, on section brownish yellow, septa appeared thickened, standing out, presenting a netlike picture. Lobules appeared sunken. Capsule was slightly shrivelled. Pancreas was poor in blood, pale brownish in colour. Splenic lymph glands slightly yellowish. Spleen measured 44 × 24 cm. Capsule was greenish yellow, the pulp was firm, trabeculae distinct, follicles just visible. Kidneys: fat capsule rich in fat, fibrous capsule slightly adherent. Parenchyma on section pale yellowish; somewhat bloodless, the pelvis yellow. Consistence was firm. Stomach distended with food; fundus normal; few gastrophilus larvae were present. Small intestines; mucosa was slightly swollen and brownish discoloured, and in parts some reddish cross stripes were present. Large intestines: slight patchy hyperaemia. Nematodes were frequent, mucosa of colon was slightly swollen. On mucosa of rectum a mucous deposit was present. The fat of the mesentery was yellow, the lymph glands were moist and yellowish. Intima of abdominal aorta was yellowish.

Intima of cranial mesenteric artery was slightly roughened. Bladder distended with clear yellow urine; mucosa had a red sprinkled appearance. A mixed thrombus was found in the vena pudenda externa. Mucosa of urethra was yellowish. Plexus chorioideus slightly oedematous and brownish coloured. Brain substance was normal. Bone marrow of humerus was fatty, that of the femur diffusely reddened.

Pathological anatomical diagnosis.—Icterus. Ecchymoses of both endocardiums. Atrophy, parenchymatous degeneration and cirrhosis of the liver; haemorrhages in bladder; thrombosis of vena pudenda; pneumonic focus; emboli in pulmonary artery; slight catarrh of intestines.

Microscopical examination.—Lower power (6x). The lobulation of the liver was indistinct. A kind of a lace-work could be made out; white narrow meshes in parts were filled in with brown tissue; there was, however, more brown than white tissue. In the white meshes were the septa; in the brown substance the small holes of the central veins could be seen. Higher power: the outlines of a lobule could be made out. The central veins contained blood. The liver cells had disappeared with but the exception of a few around the central veins and along the periphery of the lobules. The capillaries were distended and full of blood, so that the former lobule consisted principally of engorged vessels. Between these were flakes or patches of brown granular pigment, sometimes grouped around a fairly large vacuole. All the pigment gave a positive Berlin blue reaction. In Sudan-stained section a fair amount of fat was seen in what could still be recognized as liver cells; the fat granules were in groups. They were found in the centre and in the periphery. There did not appear to be an increase of connective tissue cells within the lobules, but it was well pronounced in the septa, where round cells were frequent and in clusters. The wall of the central vein seemed thickened and of hyaline and homogeneous structure.

Pathological anatomical diagnosis.—Fatty degeneration and necrosis of liver cells. Slight cirrhosis; pigment; stasis.

Case 21. Horse 10601. A five-year-old brown stallion (purchased).

Anamnesis.—Passed the intrapalpebral mallein test on the 12th September, 1916, with negative results. On the 12th September, 1916, it was submitted to a Horse-sickness experiment, being injected intrajugularly with 5 c.c. virus of horse 10219, Tzaneen 13th generation; on the 19th September, 1916, 175 c.c. serum "O." 1712 and 5 c.c. virus of horse 10036 "O." 176th generation; on the 21st 270 c.c. serum "O." 1712. The temperature was irregular; fever exacerbations were noted on three occasions, viz., on the 22nd to 104.4° F., on the 25th to 104.4° F., and on the 28th to 105° F. This horse developed an attack of contagious catarrh, which was of a rather severe nature, mucous purulent discharge being present. The catarrh ran its course at the same time as the Horse-sickness reaction.

Symptoms.—18th October, 1916. The animal showed nothing unusual on inspection at 10 a.m. At 12.30 it was reported ill and found to be leaning in the corner of the stall. Bruises and wounds above both eyes, on hips and legs were noted. It was pushing forwards violently, and it was impossible to turn it in the stall or to back it out. It was finally removed with difficulty to a loose-box and given subcutaneously 20 c.c. of 1 per cent. arecolin solution. Immediately a similar position was taken up in the loose box. In the afternoon 4 grammes of calomel were given, followed by a drink of water. 19th October, 1916.—No improvement was present in the morning, the horse standing in an unphysiological position, pushing forwards into a corner. Extensive abrasions were present on the supra orbital processes and also on the hips. The horse refused to feed; it was tucked-up; the temperature of the body was normally distributed. The animal had lost condition since it went down yesterday. 10 a.m.: The animal lay down, spread out. 10.30 a.m.: The horse did not want to stand up; an intrajugular injection of 5 grammes potassium iodine was made in 1000 c.c. physiological water. 2 p.m.: The animal was still lying spread out, no attempt was made to rise. 20th October, 1916.—7 a.m.: The horse was lying spread out, fairly quiet. Wounds above the head were marked. 9 a.m.: Animal made attempts to stand but could not keep up; it went down immediately. In the afternoon the horse was still lying spread out. In the evening it was standing and appeared attentive; it was fairly quiet and was looking for food. 21st October, 1916.—Wounds on the eyes, hips, and stifle had increased in size; bruises with swelling on forearm and knees were more marked on near side. The eyelids were very much

swollen, so that the animal could not see properly. It commenced to feed, showed general improvement. 22nd October, 1916.—The horse appeared thin and wasted. During the night it had been lying down, and was now unable to rise. Wounds on the different places had extended. Symptoms of depressions were still noticeable. 23rd October, 1916.—Bruises and wounds on left side of the body more evident than on right. Body temperature was evenly distributed and good. Consciousness to-day had improved. Marked decubitus of different parts of the body, viz., on elbows, knees, shoulders, hips, and stifles. The eyelids were still very much swollen, animal could not see well. 24th October, 1916.—Consciousness further improved. The horse was attempting to feed; it looked exhausted and morbid. The head was lowered and hind limbs were placed slightly under the body. At times the animal was observed to take up a peculiar position with the head and neck, viz., the head slightly inclined to the off side and the nose turned slightly outwards. 25th October, 1916.—Portions of the decubitus were noted commencing to slough. The horse was attentive but not feeding well. 26th October, 1916.—The feeding was fairly well, the wounds were discharging, the peculiar position of the head and neck was still assumed. The animal as far as the Staggers was concerned made a complete recovery; as far as the decubitus was concerned no cure could be effected. All wounds healed up, with the exception of those on the hips, which extended into the bones. In the near thigh abscesses developed, which extended down the tibia and burst at the proximal end of the ingastrocnemius. The horse had to be destroyed on the 2nd February, 1917.

Post-mortem report.—The condition was below fair, the abdomen was slightly distended, and rigor mortis was not present. Integument showed a healed wound above the eye and on the zygomatic ridge. A big open granulating wound was on the left tuber coxae, exposing the bone, about 15 cm. in diameter. A small wound on the trochanter and a wound in the regions of the poplitea. Blood was exuding from the nostrils (bullet). The mucous membranes of the mouth were somewhat pale. The pupillae were wide open. The anus was half open. The skin of the hypogastric region showed some bare patches. Blood of the axillary vein stained fairly well. The flesh was somewhat dark in colour and moist. The subcutaneous tissue showed no fat. The submaxillary and parotid glands showed no abnormalities. Lymph glands: mandibulars, subparotids, and upper cervicals were moist. Thyroids were pale and moist. The mucosa of the larynx was normal, a haemorrhage and a hole was noted in the left arytaenoid cartilage (bullet). The pharynx was normal. There was a wound at the base of the tongue, otherwise it was normal. The oesophagus was normal. Mucosa of trachea was normal. Peritoneal cavity contained a small amount of clear liquid. A string of connective tissue connected the tip of the caecum to the omentum. Serosa of intestines was pale, smooth, and glistening, likewise the peritoneum. Very little fat was present in the omentum and the mesentery. Diaphragm convexly forwards, on pleural side smooth and glistening, on abdominal side some rare fibrous filaments were present. In the centrum tendineum was a gelatinous substance. Costal pleura was smooth and glistening. No liquid was present in the cavities. Left apex of lung was adherent to the mediastinum. Mediastinal lymph glands were moist. Pleura of left lung was smooth, glistening; on the ventral border emphysema was present, also in the apex, the apex itself being hard. Here on section the absence of alveolar tissue was noted; it was occupied by much connective tissue. The bronchus of this portion was distended, containing pus. Left bronchus was normal. Intima of vein was smooth and glistening. Artery contained a coagulum. The lung tissue was spongy and contained little blood. A nodule was present with a thick capsule and white soft pith. The pleura of the right lung showed a few fibrous filaments on the diaphragm surface; it was smooth and glistening on the facies costalis. Right apex showed fairly extensive emphysema. A cicatrix divided the apex into two portions. Intima of pulmonary vein was smooth and glistening, artery contained a coagulum. Mucosa of thoracic trachea was smooth. Mucosa of oesophagus was normal. Intima of aorta was smooth and glistening. Lower cervical lymph glands were moist. Pericardium: the fibrosa showed a little fat, the serosa was smooth and glistening. About 50 c.c. clear liquid were present. Left atrium and left ventricles contained liquid blood and a small plasma coagulum. Left ostium admitted three fingers. Right atrium and ventricle contained liquid blood. The right ostium was open for four fingers. Epicardium contained some fat in circular and longitudinal grooves and was smooth and glistening. Left endocardium contained small coagulations under the valve. Intima of left coronary artery was smooth and glistening. Semilunar valves were normal.

Foramen ovale closed. Intima of right coronary artery was smooth and glistening. Endocardium and valves of right ventricle and pulmonary artery were normal. Myocardium on section was normal. Left wall was 3.2 cm. and the right 1.6 cm. thick. Liver: periportal lymph glands were moist. Liver was somewhat small, with sharp edges. Capsule on posterior side was smooth and glistening. Left lobe was fairly rich in blood. The lobulation was distinct, the consistence was fairly solid. Middle lobe showed on section the same texture, borders of lobules were quite distinctly marked. The right lobe was rich in blood, the lobulation was well pronounced. The centres of the lobules were dark in colour. The periphery was greyish, the septa slightly sunken. Pancreas: fairly rich in blood. Some nematode larvae were present in the thickened connective tissue. The splenic lymph glands were moist and somewhat red. Spleen appeared somewhat contracted, 34×19 cm. Capsule was smooth and glistening, it was somewhat shrivelled. On section follicles and trabeculae were distinct, the colour was brown. Supra-renal glands: the cortex of the left gland was diffusely yellow; the right gland was rather small, cortex strongly pigmented. Capsule of the left kidney was poor in fat. The fibrous capsule was adherent, on detaching it was tearing off substance. Kidney on section pale, in particular the cortex. Here were white patches. All zones could be distinguished. The consistence was fairly hard, difficult to break. The fat capsule of the right kidney contained a little fat. Fibrous capsule stripped off fairly easily; on section a dark colour of medulla and intermediary zone was notable. Cortex seemed slightly narrowed. The glomeruli were distinct. Consistence was somewhat soft. The stomach contained some food. Gastrophilus larvae were in the cardiac portion. The fundus contained a mucous deposit. A number of small tumours, size of a nut, were present, they contained pus; one of them was broken and formed an ulcer with a ring wall. The pylorus was normal. A few gastrophilus larvae were present in the duodenum. Mucosa of duodenum was normal. Small intestines: mucosa of jejunum and ileum pale. Large intestines: nematodes in caecum and colon were fairly numerous, mucosa was pale. A number of very small haemorrhages present, probably caused by nematodes. Mucosa of rectum was somewhat pale. Mesenteric lymph glands were of normal size, otherwise they were fairly moist. Intima of abdominal aorta was smooth and glistening. The lumen of the anterior mesenteric artery was somewhat widened, the intima was somewhat thickened. The bladder was empty, traces of clear urine were left, the mucosa was smooth. Urethra was smooth and glistening. The testicles were somewhat pale on section. Brain: a haemorrhage was present due to the bullet, otherwise it was rich in blood and moist. Teeth and gums were normal. The bone marrow of the humerus showed fat. Femur showed fat with a haemorrhagic focus. Proximal cancellated substance was red.

Pathological anatomical diagnosis.—Chronic interstitial pneumonia of left apex. Stasis of liver, chronic interstitial nephritis; slight anaemia.

Microscopical examination.—Liver: the lobulation was quite distinct and normal. There was no increase of connective tissue in the septa. The central portion of the lobule, the vena centralis, and the radiating capillaries contained blood, in the peripheral portion no blood was present. This gave the lobule a dark centre and a light periphery. Sudan did not show any fat in the liver cells; there were, however, small particles of fat in the cells of the bile-ducts. A little pigment could be seen in some of the liver cells surrounding the central vein. There was occasionally a small round cellular focus near a central vein or within a lobule. Kidney: the section through the white portions of the kidney showed round cellular foci in various parts, but mostly between the tubuli recti. Under the capsule at one place was seen an area in which the glomeruli were all atrophied, forming small hyaline globes. The arterial walls were much thickened, and there was a round cell infiltration present as well as an increase of fibroblastic tissue. Here some brown pigment was present. In the same section some of the tubuli recti were distended with round cells: they were pycnotic and clumped together; in other tubules the substance was homogeneous pink and showed karyorrhetic nuclear detritus. In the medullary portion was an increase of connective tissue between the tubules, forming in places small patches, and round cell infiltrations were scattered about. Here some of the tubules contained desquamated epithelial cells, their nuclei undergoing pycnosis. The thyroids showed rather large follicles filled with homogeneous substance. Lung: the interlobular septa were much thickened and in parts replaced the alveolar tissue. Only a few patches of alveoli were left; they were compressed and contained desquamated cells.

The larger bronchi showed a very wide lumen, their walls were very thick, due to connective tissue, fibroblasts and polyblasts. Pus was present in the larger and smaller bronchi; in the latter the epithelial lining had disappeared. There were round cell foci scattered throughout.

Diagnosis.—Stasis in a normal liver chronic interstitial nephritis; Bronchiectasis and interstitial pneumonia.

Epicrisis.—This horse was clinically one of acute Staggers, which is associated with acute Liver-atrophy. The horse recovered as far as the Staggers was concerned but not from the secondary complications indirectly due to it, viz., decubitus. It was destroyed. The remarkable fact was noted that the liver showed on microscopical examination no lesions; it presented a healthy picture. The conclusion that in a case of acute atrophy a liver can completely recover seems to be supported by this observation.

Case 22. Horse 10717. An aged dark brown gelding, obtained from the Defence Force. Arrived at Onderstepoort on the 11th November, 1916.

Anamnesis.—On the 13th November, 1916, it passed the mallein test with negative results. On the 15th November, 1916, it was submitted to a Horse-sickness experiment, receiving intrajugularly 5 c.c. virus of horse 10414, Tzaneen, 14th generation; on the 22nd November, 1916, 170 c.c. serum "O," 1718 and 5 c.c. virus of horse 10699 "O," 176th generation; on the 24th 255 c.c. serum "O," 1718. The temperature after this last injection was irregular, showing a fever exacerbation to 106° F. on the 29th, returning to normal on the 3rd December. After this the temperature kept normal.

Symptoms.—12th December, 1916.—The animal was found down in the morning apparently unable to rise. Fresh wounds were noted on fetlock, knees, and stifle. 9 a.m.: The animal refused to come out of the stall. The mucous membranes of the eyes were slightly congested. The animal was supported to a loose box, showing marked weakness and a staggering gait. 13th December, 1916.—The horse was noted pushing the head against the wall. 2 p.m.: The animal was knocking itself about in the stable, pushing forwards; it was semi-conscious, staggering from side to side. It got out of the box, staggering into the yard, and went down. Marked bruises and wounds were present about the head, on the shoulders, hip, stifle, and knees. The eyelids were swollen. 14th December, 1916.—6.30 a.m.: The animal was still semi-conscious; it made some attempts to feed. 16th December, 1916. The horse was attentive. It was feeding fairly well, the body was warm, both front legs were swollen. Wounds and bruises were noted on the supra orbital processes, frontal region, zygomatic arch, ears, shoulders, right knee, and off hip. 17th December, 1916.—The horse was bright and attentive, its walk was somewhat uncertain with the hind legs. 18th December, 1916.—The wounds in the frontal region reached the size of a hand. The eyelids were still swollen, also the forearms. The animal was attentive and feeding well. 20th December, 1916.—After this date the animal finally recovered. The horse was killed on the 9th May, 1917.

Post-mortem report.—The autopsy was made two hours after death. The condition was fair; rigor mortis was not complete. The abdomen was slightly distended. A bullet wound was in the frontal region. Old wounds were present on the head, a fresh abrasion on near knee. The tongue was inside the open mouth. The pupillae were distended. The blood stained well, the flesh was somewhat brown in colour. The subcutaneous tissue contained only a little fat. The mandibular lymph glands were moist, their size and colour was normal. The retropharyngeal lymph glands were moist, and some of them were haemorrhagic. The upper cervicals were also moist. There was a blood coagulum and an oedematous infiltration in the retropharyngeal tissue. The thyroids were rather small and pale. The surface of the tongue showed nothing unusual. Mucosa of the larynx, epiglottis and trachea was normal. The velum palatinum and the tonsillae were normal. The serosa of the peritoneum and of the intestines was smooth and glistening and somewhat pale. The situs viscerum was normal. The diaphragm convexly forwards. No liquid was noticed in the peritoneal cavity. The bile-duct was open. The omentum showed nothing unusual. The costal pleura was smooth and glistening. No liquid was present in the cavities. The lungs were in the stage of inspiration. The pleura was smooth and glistening. Some fibrous filaments were present on the pleura of both lobes. Emphysema was well marked on the anterior lobes. The consistence of the lung tissue was elastic and soft. The bronchus of the left lung showed a little froth, the mucosa showed nothing unusual. The intima of the artery and the vein was smooth and glistening. The right lung was

fairly rich in blood. The vein and the artery contained some semi-coagulated blood, some froth was present in the bronchus. The veins in the mucosa of the thoracic trachea were slightly injected. The oesophagus showed nothing unusual, the mucosa was normally folded. The aorta was normally elastic, the intima was smooth and glistening. The lymph glands were moist and unusually red (infiltration of blood). The wall of the pericardium contained a certain quantity of brownish coloured fat. 50 c.c. light straw-coloured transparent liquid was present in the sac. The parietal serosa was smooth and glistening. The right ventricle and atrium were slightly dilated with uncoagulated blood which escaped on opening. The right ostium was open for four fingers. In the left ventricle was a little uncoagulated blood. The left ostium was open for three fingers. A certain amount of fat was present in the transverse and longitudinal grooves. The epicardium was smooth and glistening, likewise the endocardium of the left ventricle. The bicuspidal and semi-lunar valves were normal. The right endocardium was smooth and glistening. The tricuspid and semi-lunar valves were normal. Left ventricle was 3 cm., the right 1.2 cm. thick. The intima of the coronary arteries was smooth and glistening. The myocardium was reddish brown, transparent, fairly rich in blood, the consistence was normal. The periportal lymph glands were somewhat moist and of normal appearance. The liver was somewhat small. The edges were fairly sharp. The colour of the parenchyma was light brown. The surface was smooth. A small white patch was on the capsule of the oral surface. A fair amount of blood escaped on section. A lattice-like appearance was formed by grey meshes enclosing light brown centres. The consistence of the right lobe was normal, perhaps slightly firmer than usual, but not hard. Under 6× magnification the lobules appeared normal; the meshwork was due to a greyish appearance of the periphery of the lobules, the septa were not thickened. The pancreas had a normal colouration. On section it was light brown. The consistence was normal throughout. The splenic lymph glands were normal in size and consistence. Spleen: 43 × 20 cm. The capsule was somewhat roughened due to small white nodules, the size of a millet seed, and numerous fibrous adhesions. On section the pulp was light brown. The trabeculae were fairly distinct. The follicles were hardly visible. The supra renal glands were somewhat small and pigmented in the cortex. In the capsule of the kidneys was a fair amount of fat. The fibrous capsule stripped off easily. The right kidney on section was fairly rich in blood, the zones were well marked, especially the middle zone. In the cortex the glomeruli were not visible. In the left kidney the glomeruli were visible and the intermediate zone was white. Stomach: lymph glands were pale and moist. The stomach was about half-full of food. The serosa showed nothing unusual. The mucosa showed some mucous deposit. The oesophageal portion contained *gastrophilus* larvae. In the fundus was a tumour, the size of an apple, with a caseous centre, situated near the *margo plicatus*. In the duodenum *gastrophilus* larvae were present. The small intestines showed liquid contents, the mucosa was smooth and normal in colour. The mucosa of the large intestines was smooth, slight greyish in colour. The mucosa of the rectum was normal. The mesenteric lymph glands showed nothing unusual. A certain amount of fat was present in the mesentery. The veins were injected. The lymph vessels stood out prominently. Intima of the abdominal aorta was smooth and glistening. The *arteria ileo-caeco-colica* showed an aneurism, the walls were thickened and roughened, and a few nematode larvae were present. The bladder was contracted, the mucosa showed nothing unusual. The mucosa of the urethra was smooth. Brain: extensive haemorrhages at the base due to bullet. Teeth: the first molar was slightly longer. Bone marrow: the humerus marrow was fat, lined on one side along the *substantia comparta* by red marrow. Femur: marrow was fatty, including a large patch of red marrow.

Pathological anatomical diagnosis.—Traumatism and decubitus. Brown atrophy of the skeleton and heart muscles. Slight hydropericardium. Emphysema, pleuritis fibrosa, and perihepatitis. Aneurisma parasitica. Spiroptera tumour and *gastrophilus* larvae.

Microscopical examination.—Liver: the structure appeared normal. The difference between the centre and the periphery of the lobules was in the density of the shading of the liver cells. In the centre the cell rows were somewhat separated by blood, the vein itself being filled with it, whilst in the periphery the cell rows touched each other. There was no increase in the fibrillate connective tissue structure of the septa. In some places in the septa small clusters of round cell collections were present, and also in some places in the periphery of the lobules between the liver cells containing only a few

cells in a group. In some places of the septa were eosinophiles. Some granules of brown pigment were occasionally also found in the septa. The vessels of the portal vein as well as the artery were filled with red corpuscles. Only in a very few places were there fat globules on the periphery of two adjoining lobules or an individual cell in a lobule or irregularly scattered about.

Diagnosis.—Slight stasis.

Epicrisis.—The clinical picture was that of acute Staggers, typical of acute liver-atrophy, from which the horse recovered. When killed five months later very few pathological changes were found in the liver. It is evident, therefore, that acute liver-atrophy can heal out practically without leaving any traces.

Case 23. Horse 10641. An eight-year-old bay gelding, arrived from the Defence Force on the 20th September, 1916.

Anamnesis.—On the 17th October, 1916, the horse was submitted to a Horse-sickness experiment, being injected intrajugularly with 5 c.c. virus of horse 10452, Tzaneen, 14th generation; on the 24th 150 c.c. serum "O." 1715 and 5 c.c. virus of horse 10036, "O.", 176th generation; on the 26th, 225 c.c. serum "O." 1715. The reaction was indefinite and not typical of Horse-sickness. On the 25th November, 1916, the horse was tested on its immunity with 5 c.c. virus of horse 10384 Tzaneen 107th generation, 5 c.c. virus of horse 10682, Bulawayo 14th generation; and 5 c.c. virus of horse 10689 "O.P." (122) 3rd generation. There was no reaction. After discharge from the experiment the horse was sent to the farm Doornpoort.

Symptoms.—In the afternoon of the 4th January, 1917, the horse was reported missing from Doornpoort farm, and on examination of the bordering fence it was evident that it had broken through it, traces of blood being left behind. The horse was found about two miles away and was then brought to Onderstepoort; nothing unusual was noticed in its gait—it was walking freely. The mucous membranes of the eyes, pulse, and respiration were normal. The mucous membrane of the gums showed some superficial excoriation, the lips being slightly swollen. There were scratches and wounds on the forearm, in particular of the near side and on the hind legs. The horse was placed in a loose box and given food and water. At 10 p.m. the horse was found standing in a corner of the box with the head up against the rails, rubbing the lips against the rails, and a constant movement of the mandibula was present. 5th January, 1917, at 10 a.m., the horse was standing back and not feeding, the front limbs spread apart, one in front of the other; the horse was looking back to the abdomen. Gums and tongue showed a dry surface. The horse was continually licking and drawing the lips. The pulse was 88, somewhat small; respirations numbered 30 and were slightly abdominal. The body temperature was normal. The horse received an intrajugular infusion of 500 grammes soda sulph. in 1000 c.c. water. 6th January, 1917.—The horse was feeding fairly well. Mucous membranes were slightly orange red. The horse showed general improvement; it finally recovered.

Case 24. Horse 10721. An aged dark brown gelding, obtained from the Defence Force on the 11th November, 1916.

Anamnesis.—Submitted to the mallein test on the 13th November, 1916, with negative results. On the 15th November, 1916, it was put into a Horse-sickness experiment, receiving intrajugularly 5 c.c. virus of horse 10436, Tzaneen 14th generation; on the 22nd 175 c.c. serum "O." 1714 and 5 c.c. virus of horse 10699, "O.", 176th generation; and on the 24th 265 c.c. serum "O." 1714. It had a Horse-sickness reaction from the fifth to the fifteenth day. The maximum temperature was 106° F. on the tenth day after the first injection. From the thirteenth to the nineteenth day the presence of a Dikkop was noted. The horse was sent to the farm on the 11th December, 1916. It died there on the 5th February, 1917. Examination of the skin of the carcass showed traumas that must be interpreted as those due to Staggers. It was too decomposed for post-mortem examination.

Case 25. Horse 10209. An aged dark brown gelding, obtained from the Defence Force on the 20th March, 1916.

Anamnesis.—It was submitted to a Horse-sickness experiment on the 22nd December, 1916, and received an intrajugular injection of 5 c.c. virus of horse 10436, Tzaneen 14th generation; on the 29th 235 c.c. serum "O." 1719 and 5 c.c. virus of horse 10699, "O.", 176th generation; on the 30th it received

390 c.c. serum "O" 1719. It had a reaction to Horse-sickness with remittent fever from the eighth to the fourteenth day after the first injection. The maximum temperature was 104.6° F. on the eighth day. This horse belonged to the local transport, and after discharge from immunization on the thirtieth day was put back into transport work and regularly used.

Symptoms.—On the 26th February, 1917, the foreman noticed that the horse was ill, refusing the food. Examined at 7 a.m. it showed orange-coloured conjunctivae. At 9 a.m. at inspection it could be noticed that it could not stand quiet, its head was slightly lowered, it moved around the native groom and pushed slightly forwards; standing at the rails it was noticed to yawn frequently; observed in the loose box it stood with head down in a corner. It was fairly attentive when approached. It refused all feed and remained all day in the same position. Examined at 10 p.m.: It was found in the same position. 27th February, 1917.—The horse was still in the same position this morning. It still refused food. When taken out it was seen pushing forwards more than yesterday. There were no bruises about its head. The conjunctivae were still orange-yellow. The pulse rate was 48. The abdomen was slightly tucked-up, and the respiration was increased and slightly abdominal. From this horse two not-immunized horses were infused each for 10 minutes, and the horse received 500 grammes sodium sulphate in 2000 c.c. physiological water. Towards the evening the horse showed improvement, started to feed, and no further increase in the severity of the symptoms was noted. 28th February, 1917.—The horse had been feeding, but had not finished its meal. When taken out it was less restless than yesterday, stood quietly for a while, and only subsequently began to push forwards. The conjunctiva was still yellow. 1st March, 1917.—The horse had recovered in every respect, its position and gait was normal.

Case 26. Horse 10884. An eight-year-old bay gelding, obtained from the Defence Force on the 15th December, 1916.

Anamnesis.—It was submitted to the mallein test on the 19th December, 1916, with negative results. On the 2nd January, 1917, it was put into Horse-sickness immunization, receiving intrajugularly 5 c.c. virus of mule 10712, Tzaneen, 14th generation; on the 9th 190 c.c. serum "O." 1715 and 5 c.c. virus of horse 10699, "O.", 176th generation. The following day 235 c.c. serum "O." 1715. A Horse-sickness reaction was noted from the eleventh to the twenty-second day after the first injection, with the maximum temperature of 105.6° F. on the nineteenth day. A slight Dikkop was present from the twenty-second to the twenty-seventh day. The immunity of this horse was tested with viruses obtained from horses that contracted Horse-sickness spontaneously after exposure. On the 6th February, 1917, it received an injection of 12 c.c. blood-mixture Nos. 202, 221, 222, and 225. A short abrupt reaction with an atypical fever from the sixth to the tenth day was noted. A maximum temperature of 105° F. on the seventh and eighth day was somewhat suggestive of ephemeral fever. On the seventh day six bottles were bled and injected into horses 10762 and 10985; the former showed no reaction but subsequently died of Horse-sickness due to a later injection; the latter is still alive (November, 1917). After discharge from the experiment the horse was sent to the farm and kept under observation, when it was noticed to develop Staggers on the 14th March, 1917.

Symptoms.—The horse was found down on its side on the morning of this date. It was biting the ground and grass and kicking with all legs. It was unable to rise, and when lifted could not keep on its legs. It was brought to the laboratory by float; there it was put on its legs and placed in a loose box, but it kept its position only with difficulty. It pushed immediately into the corner of the box and champed its teeth. At times the mouth was open. Transfusions were made into two horses (11013 and 11030). The horse itself was infused with hypertonic salt solution; it died at 2.30 p.m. The liver was removed immediately and an emulsion made with the Latapie apparatus. The emulsion was injected into the jugular vein of four different horses: two susceptible to Horse-sickness (11011 and 11024) and two immune to Horse-sickness (10690 and 10891).

Post-mortem report.—The condition was moderate. Rigor mortis was present. The body was still warm. The abdomen was not distended. There was some food between the teeth; the left outside incisor was broken. Integument: fresh abrasions were noted on the near side of zygomatic arch and orbital process, on knees, fetlocks, and hips. Lips and gums had a

yellowish tinge, also the conjunctivae of the eyes. The pupillae were open. The blood stained fairly well. The flesh was slightly dark. The blood was coagulated. The subcutaneous tissue was poor in fat and had a yellowish tinge. The parotid and submaxillary glands were normal. Lymph glands: the mandibulars were normal, the retro-pharyngeals and upper cervicals were slightly moist. Both thyroids were small and pale. The pharynx was normal, some froth was present in the lateral ventricle. The vessels under the epiglottis and between the rings of the trachea were injected. The right jugular vein was normal, the left showed a coagulum and some fibrous adhesions to the intima. The tongue had a dirty surface. The oesophagus was normal. The serosa of the peritoneal cavity was smooth and glistening and of a yellowish green tinge. The pleural side of the diaphragm was smooth, the peritoneal side showed fibrous filaments. There were no abnormal contents in the pleural cavities; the costal pleura was smooth and of a yellowish tinge. The bronchial lymph glands were moist and of a yellowish colour. Left lung was shrunken and poor in blood. The consistence was elastic. The pleura was shrivelled. The bronchial mucosa was normal. In the artery some coagulated blood was present and in the vein a plasma coagulum. Some emphysema was noted in the anterior lobe. The right lung was also shrunken and fairly rich in blood, and some bluish patches were under the pleura. The vessels were injected. The consistence of the lung tissue was elastic. The intima of the vein was smooth. The bronchus contained a little froth, the mucosa showed a few ecchymoses. In the artery a blood coagulum was present. Thoracic aorta: the intima was smooth and glistening. Oesophagus mucosa was folded and normal. The tracheal veins were injected. The lower cervical and anterior mediastinal lymph glands were slightly moist and yellowish. The pericardium walls contained no fat, the vessels were injected. A clear brownish yellow-tinged liquid was present (230 c.c.). The parietal serosa was smooth and glistening. The right ventricle and the atrium were distended with a coagulum of fibrin and blood. The left ventricle was contracted, containing but a small plasma coagulum and some liquid blood. A fair quantity of plasma coagula and some liquid blood were present in the atrium. The right ostium admitted four fingers, the left one three. There was a yellowish green colour fairly well developed in the fatty deposits of the epicardium. The left endocardium had a few small ecchymoses on the septum and a few on the left musculus papillaris. Ecchymoses were fairly frequent on the septum under the anterior valves. The bicuspid valves were yellowish tinged. The semi-lunar valves were normal. On the parietal wall of the right endocardium were a few ecchymoses; a slight yellowish colouration of the septum and of the tricuspid valves was present. The myocardium was of a light greyish red colour. The left wall was 4 cm. and the right $1\frac{1}{2}$ cm. in thickness. The consistence of the myocardium was softer than normal. The intima of the coronary arteries was smooth and glistening. Liver: the periportal lymph glands were moist and yellowish in colour. The liver appeared small but was rich in blood. A coagulum could be extracted from the portal vein. The caudal surface of the capsule was smooth and glistening; through it a distinct lobulation could be seen. On the cranial surface of the right lobe were numerous fibrous filaments. Otherwise the capsule was smooth and transparent. The borders were fairly sharp. On section the parenchyma appeared light yellow brown. A distinct network could be seen; greyish meshes enclosed oblong reddish spots which had sunken. The middle lobe showed similar conditions, meshes and spots appeared slightly smaller. The left lobe was similar to the right one. The consistence of the liver was fairly firm. The colour of the pancreas was normal. The splenic lymph glands were somewhat pale and moist, and slightly brown. The spleen was small, the capsule in parts was shrivelled and showed numerous small petechiae; the measurements were 40×17 cm. On section the pulp was brown. The trabeculae were distinct, the follicles were indistinct, the consistence was firm. The medulla of the right and left supra renal glands was pale, the cortex of the right one was reddish. But little fat was found in the fat capsules of the kidneys, the fibrous capsule was easily detached and the cortex was slightly pale. The medulla of the right kidney was pale, the intermediate zone was red. The consistence was normal. The medulla of the left kidney was injected and the consistence fairly firm. The stomach was distended with somewhat dry food. *Gastrophilus* larvae were present on the oesophageal portion. In the fundus the food was adherent to the mucosa, which was otherwise normal. There was a spiroptera tumour present. The pylorus was normal. The mucosa of the duodenum was covered with a slight yellowish mucus. Small intestines: the mucosa of the jejunum was light yellowish, in parts diffusely reddened. Large intestines: the mucosa of the

caecum and colon were greyish white. The mucosa of the floating colon was covered by a little yellowish mucus. The rectum contained faeces, the mucosa was pale yellow. The mesenteric lymph glands were normal. The intima of the abdominal aorta was smooth and glistening. The walls of the arteria ileo-caeco-colica were thickened and showed a roughened intima; a thrombus and larval nematodes were present. The urinary bladder was much distended; the urine was clear; the mucosa was covered with ecchymoses and petechiae, and towards the orificium with haemorrhagic patches. The urethra was normal. The veins of the pia mater were distended, the substance of the brain was moist and fairly rich in blood. The teeth were normal. The bone marrow of the humerus and of the femur was fatty with haemorrhagic foci. The nasal cavities were normal. A bloodsmear showed no abnormalities. Body weight 231½ kg., right lung 1.9 kg., left lung 1.45 kg., heart 2.3 kg., liver 3.6 kg., and spleen .75 kg. Liver: body weight= 1:64.

Pathological anatomical diagnosis.—Traumatism. Decubitus. Atrophy of liver; icterus; ecchymoses of left endocardium; cloudy degeneration of myocardium; hydropericardium; haemorrhages in the bladder. Enteritis catarrhalis. Aneurisma parasitica. Gastrophilus larvae.

Microscopical examination.—There was in some places a distinct patchy appearance, due to the absence of liver cells in the centre and in the intermediate portions of practically all the lobules. In these parts the section was transparent, no intact cells of any description being left, only a homogeneous substance in which there were a number of faintly stained round nuclei, probably belonging to destroyed liver cells and some deeper stained nuclei of round cells. In this homogeneous substance residue of liver cells could be made out, some of which contained vacuoles, others granular light brown pigment. In the periphery the liver cells were relatively intact, the staining quality of the nucleus had, however, suffered; these cells were still in a radial arrangement. The septa contained a few round cells, and these were mainly found in the vessels of the portal system, penetrating the walls and mostly collected under the intima. The disintegration of the lobules varied in intensity in the different portions of the section. In some places the lobules were but slightly affected, and only a narrow zone of destroyed cells was visible in the central portion. In the sudan-stained section fat was seen only in a few places. The light brown pigment, however, showed up well. It was irregularly distributed in the transparent portion with a tendency to accumulate in its periphery. Middle lobe: comparatively little derangement had taken place. There was only a narrow transparent zone of destroyed cells in the central portion, which was fairly rich in nuclei, the spindle-shaped and oval nuclei being quite in evidence. There was a fairly rich infiltration of round cells in the septa. Also pigment was present in the transparent portion. The sudan-stained section showed some fat globules in the narrow periphery of the transparent zone. The vessels of the portal system were filled with red corpuscles. There was practically no blood in the lobule. Left lobe: conditions were similar to those in the right lobe, the zone of destroyed liver cells was filled with red corpuscles and was, therefore, less transparent. Residues of liver cells were scattered about. Both round and spindle-shaped nuclei were present, and a fair amount of light brown pigment in granules. Only small numbers of fat globules were present, and these were mostly placed in the periphery of the transparent zone. The round cells in the periphery were mostly small round cells with deeply stained nuclei. Left kidney: the tubuli contorti showed no fat globules. A slight fatty infiltration was present in the tubuli recti of the medulla; here, also, the blood vessels were filled with blood. The right kidney showed similar conditions. Supra renal glands: the conditions in both glands were identical; extensive fatty infiltration of all three zones of the cortex, in some places more than in others, but none in the medulla. The organ was also rich in blood, particularly the medulla. Heart: the section had a patchy appearance due to the Sudan staining being taken up by groups of fibres. The staining was fairly intensive. Skeleton muscle: fairly extensive fatty degeneration of a number of fibres. One long sarcosporidium was present. Spleen: in the centre of the lymph follicles there seemed to be a certain degree of pycnosis. There was pigment present in the parts containing blood corpuscles.

Diagnosis.—Necrosis and fatty degeneration of liver cells; emigration of round cells. Pigment. Fatty degeneration of heart and skeletal muscle, of supra renal glands. Pigmented spleen; slight fatty degeneration of kidney.

Case 27. Horse 10831. An aged grey gelding, obtained from the Defence Force.

Anamnesis.—On the 19th December, 1916, it passed the mallein test with negative results. On the 3rd January, 1917, it was submitted to a Horse-sickness experiment, receiving intrajugularly an injection of 5 c.c. virus of mule 10711, Tzaneen, 14th generation; and on the 10th 220 c.c. serum "O." 1715 and 5 c.c. virus of horse 10699 "O." 176th generation; and on the 11th 280 c.c. serum "O." 1715. A Horse-sickness reaction was noted from the eleventh to the twenty-second day. Fever exacerbations to 105° F. being recorded on the fifteenth, sixteenth, and eighteenth day. On the thirty-third day the temperature was registered as 105° F., and on blood examination *Nuttallia equi* was found but in rare numbers. A slight anisocytosis was also present. The mucous membranes of the eyes were pale on that day. The horse was sent to the farm, where, on the 24th March, 1917, it was reported as suffering from Staggers.

Symptoms.—The foreman reported that the horse was noticed to be out of spirits on that day, having been dull and inattentive the previous afternoon. But he was unable to detect anything definite; the temperature was normal. On the morning of the 24th it was found outside the camp and in a wire fence, in which it was caught with its hind legs, the front legs having crossed the fence. The horse was brought back to the laboratory, where it died the same day; in the meantime a horse No. 10918 was infused with 4000 c.c. of its blood.

Post-mortem.—Autopsy was made two hours after death. The condition was fair, the abdomen normal, and rigor mortis was present. The integument showed abrasions on the left supra-orbital regions. The conjunctiva had a yellow tinge, the mucous membrane of the mouth was yellowish. The pupils were dilated. The flesh was rather dry and had a slight brownish tinge. Blood was somewhat dark with a brownish tinge, badly coagulated and rather watery. The subcutaneous tissue had a yellowish brownish tinge. The salivary glands showed no abnormalities. Lymph glands: bronchials and mediastinals were normal, also the pharyngeals, upper cervicals, and mandibulars. The thyroids were of normal appearance. The tongue, oesophagus, and pharynx were normal. The tracheal vessels were injected, the mucosa had a yellowish tinge. A few *filaria equi* were present in the peritoneal cavity. Diaphragm was normal. Pleural cavities showed no abnormal contents. Both lungs were collapsed; a few fibrous filaments were present on the pleura. On section a slight stasis was noted. Bronchus mucosa was pale, the artery normal. The thoracic aorta was normal. Pericardium contained 100 c.c. pale yellow liquid. The myocardium was pale brown and more opaque than usual and fairly firm. Left ventricle was 3.5 cm. thick and right 1.4 cm. In the left endocardium were a few echymoses. The periportal lymph glands were normal. The liver was somewhat small, the edges sharp, it felt very soft and flabby, and through the capsule a slightly mottled appearance was noticed; reddish brown and light brownish yellow areas. On closer examination it was noticed that a reddish network was mapping out the lobules, varying in colour in different areas from reddish brown through greyish brown, grey, greyish yellow to yellow. The preponderance of reddish brown or yellow gave rise to the mottled appearance. Inside this zone was a greyish area with a dark reddish point in the centre. The consistence was slightly friable. Some calcareous nodules were present. The pancreas was normal. The splenic lymph glands were slightly oedematous and enlarged. The spleen measured 44 × 20 cm. The trabeculae were very distinct. The malpighian bodies were not enlarged. The supra renal glands were rather pale. The capsule of the kidneys was easily detached. Surface of the cortex showed some of the stellate veins congested. The cortex was slightly paler brown than usual. Consistence was rather firm. Medulla had a yellowish tinge. Stomach: three larvae of *gastrophilus equi* were found in the mucosa of the oesophageal portion. The mucosa of the fundus was normal. Parasitic nodules were present in the small intestines. Mucosa was slightly pinkish, with a yellowish tinge. Sclerostomes were found in the large intestines, the mucosa was slightly swollen and yellowish. Mesenteric lymph glands were very slightly hyperaemic. In the branches of the anterior mesenteric artery a thrombus was found. The bladder was filled with pale yellow urine (slightly turbid in mass). The urethra was normal. The brain was normal. The bone marrow of the humerus was fatty. The proximate cancellated substance of the femur was slightly reddish.

Pathological anatomical diagnosis.—Traumatism. Decubitus. General icterus; hydropericardium; cloudy swellings of heart and kidneys; acute

atrophy of liver; fatty degeneration and pigmentation. Post-haemorrhagic anaemia; parasitic nodules in small intestines.

Microscopical examination.—Liver, right lobe: the normal liver picture was absent. The orientation of the former lobule was possible by the situation of the central vein and the septum. Between the two the liver cells had disappeared, or only a few were left around the central vein, and in the periphery but not in all lobules. The rest was homogeneous substance mingled with nuclei and red corpuscles. There was round cell infiltration in the septum. Middle zone: the conditions were similar to the right lobe; there was, however, more blood present, and in some lobules the destruction of liver cells was not so far advanced, viz., more relatively intact liver cells were seen. Left lobe: somewhat similar conditions as in the middle lobe; about half of a lobule was involved, and the fatty degeneration of these relatively intact cells was much in evidence. There was also a more pronounced infiltration with round cells scattered about in the destroyed lobules. Left kidney: there was a fatty infiltration of most of the epithelial lining of the tubuli contorti, but only small fat droplets were noted; it was also present in the tubuli recti. In some places in the interstitia and around the blood-vessels was a small round cellular infiltration and some of the glomeruli appeared unusually rich in nuclei. In the artery cut longitudinally the majority of the corpuscles were white. There were also some atrophied glomeruli and some with thickened capsules. Right kidney: there was about the same amount of fatty degeneration present as in the left one, but a good deal more blood. Otherwise the conditions were similar. Heart: there was a slight fat staining by Sudan in the greater number of the muscular fibres. Pancreas showed normal condition. Supra renal glands: right; a moderate amount of fatty infiltration was present in the cortex, mostly in the zona glomerulosa. Left: similar conditions. The zona fasciculata near the periphery was distinctly infiltrated.

Diagnosis.—Necrosis and fatty degeneration of liver. Emigration of round cells. Fatty degeneration of kidney and heart, muscle, and supra-renal glands.

Case 28. Horse 10943. An aged bay gelding, obtained from the Defence Force on the 21st December, 1916.

Anamnesis.—It passed the intrapalpebral mallein test on the 21st December with negative results. It was put into a Horse-sickness experiment on the 26th February, 1917, and received intrajugularly 5 c.c. virus of mule 10711 Tzaneen 14th generation; and on the 5th March 160 c.c. serum "O." 1713; and on the 7th 320 c.c. serum "O." 1713 and 5 c.c. virus of horse 10699 "O." 176th generation. A Horse-sickness reaction was noted from the thirteenth to the twenty-fifth day after the first injection. The maximum temperature was registered on the twenty-first day of 104.6° F. On the 2nd April, 1917, the horse was injected intrajugularly with 10 c.c. blood mixture Nos. 235, 252, 256, and 259 of Horse-sickness virus of horses that had contacted the disease spontaneously. On the morning of the seventh day it was bled. In the evening the temperature registered 106° F. The pulse was intermittent. On the eleventh day the mucous membranes were pale, the next day they had a yellowish tinge. The horse recovered and was sent to Doornpoort.

Symptoms.—24th May, 1917.—The horse running at Doornpoort was noticed by the foreman to-day at 2 p.m. to be ill. It sweated profusely, its penis was hanging out, and the jaws were kept moving. The horse turned in a circle to the right with the head bent inside. It was then secured and placed in the float to be sent to Onderstepoort. It arrived here at 4 p.m. lying in the float. It had to be dragged out and was put on its legs. It was then able to walk with assistance into the loose-box. Here it adopted at once a pushing position; the head down to the ground, the neck and shoulder against the manger, and so violently was the pushing kept up that both hind and front legs slipped, and finally the horse landed on the floor. It was now dragged into a more comfortable position and left alone. It then kept quiet, and at 6 p.m. was still in the same position. After being placed in the box the horse passed urine of a transparent but dark colour. At 10.30 the horse was up and standing in the middle of the box without support. It kept its lips constantly moving, having some hay between them. After removing the hay the movement of the lips, a kind of drawing in, continued. The pulse was increased and full. The skin of the horse on touch felt a little cold. 25th May, 1917.—The horse was still up and in somewhat the same position as the previous evening. The lips were still slightly moving. It now

showed fresh bruises in the supra orbital regions which it did not have on its arrival from Doornpoort. At 10.15 a.m. some of its blood was infused into horse 11327, and at midday into horse 11340. The former was infused for ten minutes, the latter for four minutes, when it showed symptoms of shock, and the infusion was discontinued. At 2 p.m. the horse 10943 was on its feet, with head bent backward and sideways and the front legs spread apart, pushing forwards and leaning towards the corner of the box. A few minutes later it was down in a sternal position, the head hanging low. At 2.45 p.m. it was infused with 100 gr. dihydrogen phosphate in 500 c.c. water. The horse, which was lifted up for the infusion, went down again, and now remained stretched out on its side. At 6, 8, and 10 p.m. the horse was found in the same position. It died during the night. Urine analysis: Albumen 0.4 per cent. (4 per 1000); total nitrogen 1.2 per cent.; NH_3 —Nitrogen 0.12 per cent. = 10 per cent. of total nitrogen (but urine was some hours old); amino acids: trace only; blood pigments: oxyhaemoglobin and methaemoglobin present in large amount; bile reaction present.

Post-mortem.—The autopsy was made about twelve hours after death. The condition was fair. The abdomen slightly tympanitic. Rigor mortis was present. The integument showed fresh abrasions above eyes on both sides. The tongue was kept inside the mouth; the mucous membrane was pale yellowish. The anus was slightly open. The blood was somewhat dark, it stained well. On removing the skin the subcutaneous tissue and aponeuroses appeared yellow with an orange tinge. The muscles were somewhat dry, resembling the colour of terra cotta. The right parotid gland was somewhat moist and dark brown, the left somewhat pale. The subparotid lymph glands were enlarged, dark, haemorrhagic with yellowish tinge. The upper cervical lymph glands were moist with oedematous infiltration. The mandibular lymph glands were moist and slightly enlarged, the surrounding tissue was moist. The submaxillary glands showed no abnormalities. Some mucus was present at the base of the tongue, at the tip were some abrasions. The mucosa of the guttural pouches was injected. The tonsillae and the oesophagus showed nothing abnormal. The pharyngeal walls were somewhat thickened by oedematous infiltration. Peritoneal cavity: the situs viscerum was normal. The large intestines were distended with gas, also the stomach. The parietal and visceral serosa of the peritoneal cavity showed a yellow tinge. Filaria was present in the peritoneal cavity. The diaphragm was convexly forwards, the pleural side was smooth, the peritoneal side showed some fibrous filaments. No foreign contents were present in the pleural cavities, the pleura was yellowish. The mediastinal and bronchial lymph glands were yellowish and somewhat moist. The cervical trachea contained some mucus and foam, and at the lower end some froth. The veins were somewhat injected. The mucosa had a slightly yellowish tinge with a general reddish longitudinal striation. Intima of thoracic aorta was smooth and glistening. *Lymphoglandulae cervicales et mediastinales anteriores* were much enlarged and black in colour; the supporting connective tissue above the arcus aorta was gelatinous. Both lungs were half collapsed. The pleura was smooth and glistening, with a bluish tinge (stasis). Some emphysema was present in both anterior lobes. There was some enlargement of septa along the border of the left lung due to infiltration, with clear yellowish liquid. Its consistence was elastic. The vein was empty, the intima was yellowish. The bronchus contained some gelatinous substance and froth. The right lung on section was found rich in blood, the parenchyma dark in colour. The vein contained some partly coagulated blood, the intima was smooth and glistening. The mucosa of the bronchus was yellowish, slightly injected; the artery also contained some partially coagulated blood; the intima was smooth. Pericardium contained 100 c.c. of haemoglobin stained, dark brown transparent liquid. The right ventricle was empty but not contracted, in the atrium was a small amount of blood; the ostium admitted a hand. The left ventricle was contracted and contained but little coagulated blood. In the atrium was cruor and a plasma coagulum. The ostium admitted three fingers. A fair amount of fat was in the transverse and longitudinal grooves. The epicardium was smooth and glistening; it contained a few haemorrhages at the base of both ventricles. The apex cordis had a greyish appearance. The right endocardium was pale, on the parietal side a few echymoses and on left musculus papillaris. Both tricuspid valves and semilunars were normal. In the left ventricle fairly diffuse haemorrhagic extravasations were found in the muscoli papillares, also in the trabeculae carnaeae. The bicuspid valves were normal. Myocardium: left wall was 3.5 cm. and right 1.5 cm. in thickness. The intima of the coronary artery was smooth and

glistening. The septum had a somewhat boiled appearance. The periportal lymph glands were moist and slightly yellowish. The liver appeared rather small and the borders were sharp, the colour was somewhat dark. A few fibrous filaments were present on posterior surface, the anterior one was smooth. On section of the right lobe blood escaped freely. A peculiar meshwork was noted. The centres of the lobules were dark in colour and sunken. On section of the middle and the left lobe were seen some large yellowish areas mingled with red areas. The parenchyma on section appeared somewhat glossy and moist; the consistence was fairly firm. The pancreas was somewhat moist, the consistence was normal. The splenic lymph glands were somewhat enlarged, yellowish brown. Spleen measurements were 25×46 cm. In parts the capsule was shrivelled and in parts drawn in. The parenchyma was dark brown, the consistence somewhat firm. The trabeculae were just visible, the follicles were not visible. The medulla of the right supra renal gland showed black haemorrhages. The left showed injection of the medulla. The cortex was yellow. The right kidney capsule had a fair amount of fat. The fibrous capsule stripped easily. The parenchyma had a pale brown appearance; on section it was moist, the cortex somewhat opaque. In the intermediate zone were yellow and red streaks. The left capsule stripped easily. The parenchyma had a brown yellow tinge and was fairly rich in blood. On section the cortex appeared thickened, the intermediate zone yellow, with red stripes. The consistence was somewhat friable. The stomach was distended. The mucosa was diffusely reddened. A mucous deposit was present. Only a few young gastrophilus larvae were found in the oesophageal portion and in the duodenum. The jejunum and ileum were diffusely slate coloured. The mucosa of the rectum was diffusely injected. The mucosa of the caecum and colon were greyish discoloured, the floating colon showed haemorrhagic patches. The mesenteric lymph glands were moist and slightly enlarged. The mesentery had a fair amount of fat, yellowish discoloured. The intima of the abdominal aorta was smooth and glistening. The arteria ileo-caeco-colica contained an aneurism with thickened walls. The urine was viscous, brown, muddy. The mucosa of the bladder was injected and showed numerous petechiae. Brain had a somewhat moist appearance, and was poor in blood; the cerebellum was the same. The bone marrow of the femur was red in lower and yellow in upper half. The humerus showed red marrow rather extensively in the shaft. Teeth showed no abnormalities. Body weight 274.6 kg., right lung 3.4 kg., left lung 2.5 kg., heart 2.5 kg., liver 3.2 kg., spleen 1.5 kg., left kidney 0.6 kg., right 0.6 kg. Liver: body weight = 1:86.

Pathological anatomical diagnosis.—Icterus; acute atrophy and fatty degeneration and pigmentation of the liver; fatty degeneration of the kidneys; slight tumour splenis; fatty degeneration of the muscles of the heart and the skeletal muscles. Anaemia of the brain. Ecchymoses of left ventricle. Oedema pulmonum. Hyperaemia of stomach and intestines; haemorrhagic and oedematous infiltration of lymph glands.

Microscopical examination.—There was practically no liver tissue left. The lobule was occupied by homogeneous debris, in which no structure could be made out. Only occasionally in the periphery were a few liver cells seen vacuolated and torn. In some places were also newly formed bile-ducts, their cells were also vacuolated. The fat stain showed a great number of very large fat drops distributed in the lobules. There was also some light brown pigment present. In the septa was also a round cellular infiltration scattered about. Kidney: in the tubuli contorti and also in the tubuli recti was an extensive fatty degeneration present. In some of the tubules was a blackish substance, which consisted of black rods and granules of different size staining very deeply, the granules being derived from nuclear substance.

Diagnosis.—Liver: fatty degeneration, necrosis, pigment, regeneration of bile-ducts. Kidney: fatty degeneration and necrosis.

Case 29. Horse 10854. An eight-year-old grey gelding obtained from the Defence Force on the 15th December, 1916.

Anamnesis.—The horse passed the mallein test on the 19th December, 1916, with negative results. On the 30th January, 1917, it was submitted to a Horse-sickness experiment, receiving an intrajugular injection of 5 c.c. virus of horse 10436, Tzaneen 14th generation; on the 5th of 160 c.c. serum "O," 1715 and 5 c.c. virus of horse 10699 "O," 176th generation; and on the 6th

of 265 c.c. serum "O." 1715. A Horse-sickness reaction was noted from the sixth to the thirteenth day, the maximum temperature during this period being registered on the seventh and eighth day at 104° F. A Dikkop was noted on the fifteenth day, but had gone by the twenty-first day. On the 2nd March, 1917, the horse was sent to Doornpoort.

Symptoms.—On the 3rd April, 1917, the horse was found down alongside the fence in Doornpoort unable to stand. Nothing amiss was noted the night before. The horse was stretched out, kicking and biting the ground. It was brought to Onderstepoort in the morning of the 4th and 4000 c.c. of its blood were infused into horses 11057 and 11055. The horse was then killed.

Post-mortem.—The autopsy was made two hours after death. The condition was fairly good, the abdomen slightly distended. Decubitus on left haunch, elbow, and shoulder joints. Numerous transverse scratches on forehead, left supra orbital region and face, some of them going through the skin into the subcutaneous tissue. Inside of the lips, which were pendulous, and on tongue, which protruded from mouth, was a deposit of clay and grass. Inside both lips the mucous membrane was badly abraded and appeared to be due to biting by the incisor teeth. Both nostrils contained a little earthy material. The conjunctiva of the left eye had a pale yellowish tinge. The penis was hanging from the sheath. The anus showed nothing abnormal. The abdomen and left side were covered with mud. Subcutaneous tissue showed light yellow colouration. The muscular tissue was somewhat pale with a brown hue, the aponeuroses and fasciae standing out yellowish. The fatty tissues were ochre yellowish in colour. The salivary glands showed nothing unusual. The mandibular and retro-pharyngeal lymph glands showed nothing unusual. Intima of carotid artery and jugular vein were normal. An earthy deposit was on the tongue, the tip slightly excoriated; the substance had a faint yellowish tinge. Mucosa of the oesophagus appeared normal. The pharynx contained a small quantity of dirty brownish deposit, the mucosa showed nothing unusual. Larynx: slight blood-tinged deposit on the mucous membrane of the epiglottis. Trachea: semi-coagulated blood-tinged fluid was present along entire surface. The mucous membrane showed the small vessels congested. The oral surface of the diaphragm showed nothing unusual. Fibrous filaments were along the muscular portion of the caudal surface. The left pleural cavity contained no excess of liquid. The cortical portion of the bronchial and mediastinal glands was yellowish in colour. Both lungs were almost collapsed. The pleura of the left showed nothing unusual. On section the parenchyma was brownish in colour, with a mottled appearance in parts. On pressure blood appeared scantily and no oedema was present. The bronchi showed nothing unusual, the pulmonary vessels were normal. The anterior lobe of the right lung showed a few subpleural haemorrhagic points and also some emphysema. The parenchyma was fairly rich in blood. It was mottled and more brown than the left. The bronchi contained some coagulated blood. The pulmonary vessels showed nothing unusual. The pericardium contained 75 c.c. clear straw-coloured liquid. The right ventricle was full of semi-coagulated blood, and a small amount of fibrin coagulum was present. The ostium admitted a hand. The left ventricle contained a fibrin clot. The atrium was empty, the ostium was tight for four fingers. Epicardium: ecchymoses were present along the coronary grooves particularly on the right side; a few were also on the auricles. The coronary vessels stood out distinctly. The coloration was light brown with a white hazy striation. The left wall was 4 cm. thick, the right 1.5 cm. The myocardium was pale greyish brown in colour, almost grey. The left endocardium showed profuse ecchymoses and extravasations, they were more marked along the valves. In the right endocardium only a few haemorrhages were present. A faint yellowish coloration was noticeable. The periportal lymph glands were swollen, on section showed congestion and oedema. The liver was small and shrunken, borders were very sharp, and in parts the capsule extended beyond the parenchyma. The left and right lobe were about the same size, the middle lobe was comparatively large, the colour greyish yellow brown. In parts grey predominated, in parts the brown and in other parts the yellow. The consistence was firm and leather-like. On section the left lobe was more yellow than the right. The dilated central vein of a lobule could be seen standing out clearly and surrounded by a yellowish zone, which again was surrounded by a reddish zone. The periphery was grey, and within this grey zone the sunken septa could occasionally be seen. On both surfaces a small number, about one dozen, whitish yellow points (size of a millet seed to bean) were present. On section they appeared to be centres of necrosis and surrounded by a white fibrous capsule. The smaller

ones had a homogeneous white appearance. The splenic glands and pancreas showed nothing unusual. Spleen measured 40×18 cm. The capsule was light grey in colour. The consistence was fairly hard. On section the parenchyma was reddish brown in colour. The trabeculae were very distinct. Malpighian bodies were not prominent. Supra renal glands: left 8.5×2.8 cm. Cortex yellow, the medulla stood out as a grey zone. Right 10×2.5 cm. Cortex and medulla were very distinct, the cortex had a yellow tinge. The right kidney capsule had a fair amount of fat, the fibrous capsule was easily detached. On section the cortex showed the glomeruli very distinctly; the parenchyma was somewhat yellowish. The medulla showed nothing unusual. The left kidney was the same as the right. Fundus of the stomach was hyperaemic, well marked in the centre, more patchy on the periphery. Large numbers of spiroptera were present. The oesophageal portion contained a number of gastrophilus larvae. In the mucosa of the duodenum was a slight arborescent congestion. The mucosa of the small intestines was yellowish in colour, with a thick mucous deposit on the surface. Large intestines: the mucous membrane was slightly swollen, nematodes were present in scanty numbers. The small colon had a yellowish-tinged mucosa with a sticky mucous deposit on surface. The rectum was distended with hard balls of faeces coated with mucus. The mucosa was pale. The mesenteric lymph glands showed nothing unusual. The mesentery was rich in fat of an ochre yellowish colour. The branches of the mesenteric artery contained thrombi, nematode larvae being adherent to surface and surrounded by a fibrin clot. The bladder was fairly distended with urine. The mucosa was ecchymosed throughout the entire surface. Haemorrhagic spots were present, varying in size from a pin's point to large pin's head. Brain: there appeared to be a congestion of the cerebral membrane. All the vessels were very distinct. The teeth were covered with earth and chopped food and uniformly worn. Bone marrow in the humerus was of a distinct yellowish colour, it was normal in consistence. The femur: the upper epiphysis was haemorrhagic, deep red in colour, the rest had a yellowish appearance. Body weight 283.7 kg., right lung 1.8 kg., left lung 1.3 kg., heart 2.3 kg., liver 4.25 kg., spleen 1.2 kg., and kidneys 1.35 kg. Liver: body weight = 1:66.

Pathological diagnosis.—Decubitus. Icterus. Stasis and atrophy of liver; fatty degeneration of heart; trauma of tongue; oedema of lymph glands; haemorrhages in lungs; fatty degeneration of kidneys; enteritis catarrhalis, atrophy of spleen; parasitic nodules.

Microscopical examination.—The normal lobular picture had disappeared. As landmarks of a lobule the central vein and the interlobular septa could be recognized. The former showed up in many parts as a hyaline ring-shaped or oval-shaped or longitudinal band according as the wall was cut. The septa showed up by their fibrillar bundles, which in some places appeared abnormally thick and were hyaline. The vessels contained blood shades. The bile-ducts stood out distinctly. Round cells were present more in some septa than in others, not to any great extent, except at one place, where a number of septa showed both a round cell and eosinophile cell infiltration and the septa were thickened by fibrillar bundles, between which the cells were placed. In some places a light brown pigment was present in the cells. The place formerly occupied by the radiating liver cell rows showed in the most advanced stages a foam-like structure, consisting of vacuoles in a homogeneous ground substance in which only a few nuclei were scattered about. In most lobules along the septa parts of liver cell rows could be made out, but only in a few cases could intact liver cells be recognized, and these were then placed in the most peripheral portion of the lobule. At one place of the section the foamy structure contained homogeneous substance of a pinkish staining (serum), and here at some places newly formed bile-ducts could be made out by their lighter colour—they radiated into the periphery of the lobule. It was under the capsule that these regenerative processes were most pronounced. In the sudan-stained section the presence of fat was well indicated; it was either placed ring-like in the periphery of the lobules, in some places scattered in the lobules and occasionally around the central veins. Also the newly formed bile-ducts showed fine granules of fat. In this section the light-brown pigment showed up well. Left lobe: the conditions were similar to those in the right lobe; the foamy structure here, however, was soaked with a homogeneous pinkish substance. The vessels were filled with blood shades. Middle lobe: similar changes as in the left and right lobe. Whilst the nuclei in the former portion were absent in the foamy structure, here they were present. Some of them were spindle shaped, other were round. The former were endothelial

cells, the latter partially liver cell nuclei, in which case the outline of a liver cell could be made out, or leucocytes, including some polymorphonuclears. The destruction of the lobules was not so far advanced as in the former two sections. Right kidney: Sudan stain. In the tubuli contorti and the tubuli recti (Henle's loop) the epithelial cells were crammed full of fat globules. The glomeruli were entirely intact. In the haematoxylin-eosine stained section there was some vacuolation present, but nothing to indicate the extensive changes as revealed by the fat stain. In some of the tubuli contorti the nuclei appeared pycnotic and the plasma stained rather deeply. The larger vessels were engorged. In some places between the tubuli contorti was a scanty small cellular infiltration. Left kidney: In the pars convoluta were similar conditions. In the intermediary zone the narrow and thick Henle's loops showed fat, not the large tubuli uriniferi. Heart: right, the section was cut crossways and the fibres were all uniformly dusted with very fine fat granules. Purkinje's fibres were red and crammed with small fat globules. Left: longitudinal section. The fibres showed scattered small fat globules, more in some than in others. Muscle: Sudan-stained fibres showed intensive accumulation of fat droplets, so that the muscular structure became obscured; there were a few which were less affected. They were running usually alone, rarely two or more alongside the lesions, so that stained and unstained fibres were alternating. Spleen: there was much pigment present in the sinuses. There was some pycnosis and karyorrhexis in the germinative centres.

Diagnosis.—Atrophy, necrosis, and fatty degeneration of the liver cells; pigment; emigration of round cells; eosinophilia; regeneration of bile-ducts; fatty degeneration of the tubuli contorti and tubuli recti; fatty degeneration of the heart and the skeleton muscle. Pigmentation of the spleen and commencing necrosis of the germinal centre.

Case 30. Horse 10814. An aged chestnut gelding, passed the mallein test on the 19th December, 1916, with negative results.

Anamnesis.—On the 2nd January, 1917, it was submitted to a Horse-sickness experiment, and received an intrajugular injection of 5 c.c. virus of horse 10436 Tzaneen 14th generation; on the 9th 5 c.c. virus of horse 10699 "O.", 176th generation, and 240 c.c. serum "O." 1715; and again on the 10th of 240 c.c. serum "O." 1715. A Horse-sickness reaction took place from the sixth to the fourteenth day after the first injection, the maximum temperature of 104° F. being registered on the eighth, ninth, and eleventh days. The horse was sent to Doornpoort for further observation.

Symptoms.—28th March, 1917.—The horse was reported to show symptoms of Staggers this morning. It was found tied up in the fence; it was noted not to have been well the night before. It was carted to the laboratory but died before arrival.

Post-mortem.—The condition was fair; the abdomen was slightly distended. The integument showed wounds in supra orbital and frontal regions and on the hips. Mesogastric region was wet with urine. The natural openings showed nothing unusual. The conjunctiva was pale. The subcutaneous tissue was rather dry and the flesh pale red. Lymph glands: the bronchials were hyperaemic and oedematous, also the mediastinals, the mandibulars were slightly oedematous, the posterior pharyngeals were slightly hyperaemic, the lower cervicals and anterior mediastinals were hyperaemic and oedematous. The thyroids showed nothing unusual. The parenchyma of the tongue was yellowish, the oesophagus showed nothing unusual. In the pharynx was a small amount of foam. The trachea was slightly diffusely hyperaemic. In the larynx was a small amount of mucus, the vessels of the epiglottis were injected. The visceral serosa of the peritoneal cavity showed a few fibrous filaments. The diaphragm was convexly forwards, fibrous filaments were present on the caudal surface. The left pleural cavity contained 50 c.c. clear yellow liquid. A few blood extravasations were on the dorsum of the pleural walls. The thoracic trachea showed slight diffuse hyperaemia. The aorta and the oesophagus showed nothing unusual. The left lung was partially collapsed, the pleura smooth and glistening. On section the parenchyma showed nothing unusual; the mucosa of the bronchus was slightly pale. The pulmonary artery and vein showed no abnormalities. The right lung was half collapsed, elastic. The pleura showed nothing unusual. On section the tissue showed no abnormalities. The bronchial mucosa was slightly pale. A few small

haemorrhagic extravasations on the pleura of the pericardium. Cavum pericardii contained 25 c.c. clear liquid. The right ventricle was slightly dilated, the ostium was tight for the fist and the left admitted a hand. Both ventricles contained dark non-coagulated blood. Epicardium: a few petechiae and small ecchymoses were in the right coronary grooves. Right endocardium: a few slight extravasations were on papillary muscles. Left endocardium: large patches of ecchymoses were present, more marked on the papillary muscles. Right wall, 1 cm., left wall 3.5 cm. in thickness. On section the myocardium rather pale and slightly yellowish; the consistence showed nothing unusual. Periportal lymph glands were enlarged, slightly hyperaemic, and oedematous. The liver was rather small, the consistence fairly tough. Numerous small white nodules were found under the capsule. The edges of the liver were sharp. The capsule was smooth. On section the parenchyma was mottled, brown and yellow. Some lobulation could be recognized. There were ragged yellow outlines in the periphery of the lobule, circumscribing a small dark area, with sometimes a little dot in its centre. The pancreas showed nothing unusual. The splenic lymph glands were slightly hyperaemic and slightly oedematous. The edges of the spleen were slightly rounded, the consistence was firm. On section the trabeculae were not very distinct. Malpighian bodies were fairly distinct. Suprarenal glands; the cortex was fairly rich in fat. The capsule of the left kidney was easily detached, the vessels under the capsule were slightly injected. On section the cortex was slightly brownish, the glomeruli were distinct. The boundary and medullary zones were slightly hyperaemic. The consistence was normal. The right capsule was slightly adherent, the vessels of the capsule were injected. On section the cortex was brownish, the medulla slightly hyperaemic. The stomach was distended with firm contents. The mucosa of the fundus had a patchy hyperaemia. A few gastrophilus larvae were present in the oesophageal portion. A small amount of dark brown contents were present in the jejunum, and a small amount of rather solid contents, small pellets or balls, in the ileum. Patches of marked hyperaemia were found in the ileum. The mucosa of the jejunum showed patchy hyperaemia and slight yellowish discoloration. The mucosa of the caecum was slightly thickened, and large hyperaemic patches were present. The colon had a slight slate discoloration and contained a few sclerostomes. The mesenteric lymph glands were slightly swollen and oedematous. The mesentery was rather pale. The abdominal aorta showed nothing unusual. In the branches of the anterior mesenteric artery were a thrombus and nematode larvae. The bladder was distended, containing 2 litres of a slightly brownish urine. There were some haemorrhagic extravasations in the submucosa and some petechiae. Brain, cerebral cavity: a slight excess of liquid and the vessels of pia mater were slightly injected. The teeth were regular. The bone marrow of the humerus had a fatty appearance, in the periphery was a haemorrhagic infiltration. The femur showed haemorrhagic infiltrations. Body weight 272.4 kg., right lung 1.8 kg., left lung 1.5 kg., heart 1.9 kg., spleen 1 kg., kidneys 1.12 kg., and liver 3.2 kg. Liver weight: body weight=1: 85.

Urine Examination.	Urine of Horse 10814.	Urine of Normal Horse.
	Per cent.	Per cent.
Total nitrogen	0.448	0.70
Ammonia nitrogen (=6.3 % of total)	0.028	0.006
Amino acids	trace	trace
Acetone bodies	normal	normal (0.02 %)
Albumen... ..	trace	trace
Glucose	absent	absent
Bile pigments	A good "Huppert" reaction on 10 c.c. of urine, bile distinctly present	
		trace

Apart from the presence of an amount of bile pigment rather above the normal (not high, however) the only abnormality was in the proportion of ammonia. Since the urine examined was only a sample, the 24-hour excretion could not be given, but from the creatinin ratio there was nearly seven times as much as in the normal control. The proportion of the total nitrogen made up by ammonia was 6.3 per cent.; this was above what would be considered normal, but one might have expected it much higher.

Pathological anatomical diagnosis.—Slight hydropericardium: ecchymoses of epicardium and both endocardiums; atrophy of the liver; fatty degeneration and pigmentation; fatty degeneration of kidneys; slight general icterus; ecchymoses in bladder; hyperaemia of stomach and intestines.

Microscopical examination.—Liver: the greater portion of the lobules of the right lobe were occupied by blood, leaving a small zone around the central vein, where parenchyma cells could be recognized, and in the periphery a patchy distribution of budding bile-ducts. The liver cells around the central veins were by no means intact, although they could still be recognized as cells. They were vacuolated and in Sudan-stained sections showed to possess fat globules. In the large portion occupied by blood no liver cells could be made out, although nuclei were fairly frequent, both round and oval ones, the former of the white corpuscles in the blood, the latter of the endothelial cells of the capillaries. In the Sudan-stained section no fat or only rarely a droplet was found within this portion, the droplets stopped short at the inner periphery of the portion. In the periphery the budding bile-ducts were present, forming in patches pieces of cell tubes, sometimes with a distinct lumen and a regularly arranged epithelial lining; in some cases a cell-bud full of vesicular nuclei without any distinct differentiation of the cytoplasm was noted. This kind of bud entered the periphery of the lobule; in other buds the nuclei were arranged in the periphery and a differentiation of cells was demonstrable. In the septa and between the budding bile-ducts were round cells, very abundant in some places and less in others; they were also found to infiltrate the walls of the portal veins and to form small plugs in smaller vessels, the plug consisting entirely of these cells. Two kinds of cells were present, a smaller with a compact nucleus and a larger with a more loose nucleus. The central and sublobular vein contained much blood, and the walls of the latter appeared in places unusually thick and hyaline. In this section were also small nodules, 480 to 640 μ in diameter, the centre of which consisted of epithelioid cells surrounded by round cells and fibroblasts, the latter concentrically arranged. There were a fair number of eosinophiles in the peripheral portion of the capsule. There were also giant cells present in some centres and in the periphery of the epithelioid zone. These nodules were in the septa of the lobules and bile-ducts showed up in their periphery. In the middle lobe the general arrangement was as in the right lobe, but, curiously enough, there were no budding bile-ducts in the periphery, which, however, was richly infiltrated with round cells. Relatively complete liver cells were seen bordering the central vein and the septa, so that the zone occupied by blood was comparatively narrow and formed a red ring around the middle portion of the lobule, being in many lobules completely isolated, whilst in others the periphery was touching an adjacent one. The liver cells contained fat only to a small extent. In the left lobe conditions were also somewhat different. There was a considerable zone of relatively intact liver cells around the central vein, possessing much fat; there was also an infiltration of the septa with white corpuscles, but to a lesser degree than either in right or middle lobe, and no budding bile-ducts were present. Kidneys.—Right: in the epithelial lining of the tubuli contorti were a fair number of fat droplets; practically all the tubuli contorti and some tubuli recti showed them. In some parts there was a round cellular collection between the tubules and some glomeruli were atrophied. One of these round cell clusters showed a centre of epithelioid cells. Some of the glomeruli showed a fairly thick capsule, and the tuft inside appeared smaller than usual. There was a fair amount of blood in the medulla and intermediate zone. Left: a similar fatty degeneration was present in the epithelial lining of the tubuli contorti and also in a greater number of the tubuli recti. It was also present in the thinner tubes of the Henle's loop. Similar round cell collections and atrophied glomeruli were in the right kidney. Heart: the fibres of the left and right ventricle as well as of the septum showed a fine dusting with fat globules. Skeleton muscle: some of the fibres showed a fine dusting with minute fat droplets, but the striation was in no way obscured. Spleen: there was much dark-coloured pigment present and mostly in the spaces occupied by the blood. It was not present in the spaces where the

white cells were dominant. The centres of some of the lymph follicles appeared to possess pycnotic nuclei. Lungs: nothing unusual. The capillaries were fairly distinct by injection with red corpuscles. Suprarenal glands: in the zona fasciculata almost all tubules showed fat droplets in the cells and of different intensity, so that in parts there was a patchy appearance owing to the irregular distribution of the fatty degeneration. Also the zona glomerulosa was rich in fat, but not in all parts. The conditions in both glands were identical.

Diagnosis.—Necrosis, stasis, and fatty degeneration of liver cells. Regeneration of bile-ducts. Fatty degeneration and chronic nephritis. Fatty degeneration of heart and skeletal muscle. Fatty degeneration of the suprarenal glands.

Case 31. Horse 11046. An old bay gelding bought on the 29th March, 1917.

Anamnesis.—On the 30th March, 1917, it was submitted to the mallein test with negative results. On the 10th April, 1917, it received an intrajugular injection of 200 c.c. serum "O." 1715 and on the 12th of 325 c.c. serum "O." 1715. No reaction was noted after these injections. The horse was sent to the farm Doornpoort.

Symptoms.—On the afternoon of the 16th June, 1917, it was noted to be ill; it looked dull, not feeding well, and the conjunctival mucous membrane showed a trace of orange. The horse was brought to the laboratory on the 17th June, 1917, about 7 p.m., when it appeared to be somewhat restless and pulling on the riems of the halter. The temperature was 101° F., the pulse somewhat soft, the conjunctival mucous membrane orange tinged. The horse appeared, however, to be quite conscious. It was placed in a loose box. On the 18th June, 1917, it had the tongue between the teeth and was pushing the head against the wall. The pulse was 48. The heart impulse was not strong and slightly extended. Respirations 14 per minute. Wounds were noted on the supra-orbital ridges. When taken outside the horse moved occasionally in a circle; it showed slight opisthotonus; it was not feeding. The eyes were apathetic, and no movement was seen in eyelids. The front limbs were spread out. The horse was walking as if blind. At 11 a.m. some blood of this horse was transfused into horse 11326 for four minutes, and horse 11324 also for four minutes, and two litres were removed for serum. 2.30 p.m.: The stupor was more marked, the weakness was further increased, the horse swayed from side to side. It was bled another two litres. It received then an intrajugular injection of 120 grm. dihydrogen sodium phosphate in 1000 c.c. physiological water. After this injection it showed peculiar yawning movements, and every now and then threw the head upwards and to one side, rubbing the whitewash off the wall. These movements were kept up for fully 1½ hours; the horse remained standing in one position, leaning against the wall. 19th June, 1917.—Nose and upper lip were swollen. The area of the heart impulse had extended, impulse not strong. The pulse was imperceptible, the heart-beats numbered 96. Bruises were present on frontal region and excoriations in fossa temporalis. The eyes were closed, there was some mucous discharge present. Both eyelids were swollen. The horse was dull and quiet. Respiration was 10. The horse was easily led when taken out; it went down during the night. 20th June, 1917.—It was standing in a sleepy position. At 10 a.m. the horse was found under the manger, struggling to get up, creeping forward like a dog, unable to regain the standing position. The horse was killed the same day. Urine analysis on the 18th June, 1917.—Total nitrogen 0.696 per cent.; ammonia 0.015 per cent. = 2 per cent. of total nitrogen; amino acids 0.074 per cent. = about 11 per cent. total nitrogen; albumen = normal (practically absent); bile pigment = trace; 19th June, 1917.—First sample passed overnight of 18th-19th was practically the same as that of the 18th. Second sample passed during the day: ammonia and amino acids practically the same as on 18th. Small amount of albumen now present, and the bile pigment reaction (Huppert), though still slight, was more marked than on 18th (positive distinct). The chief abnormality in these urines was the large amount of amino acids. This was probably tryosine, but it is difficult to be quite certain since no crystals could be found in the deposit in standing or concentrating. 20th June, 1917.—Pre-mortal urine: bile reaction positive distinct; higher than any of the three preceding samples; albumen 0.66 per cent.; total nitrogen 0.742 per cent.; ammonia nitrogen 0.36 per cent. = approximately 5 per cent. of total nitrogen; amino acids = 0.082 per cent. = approximately 11 per cent. of total nitrogen. (Similar to urine of 18th.)

Post-mortem report.—The autopsy was made one hour after death. Rigor mortis was not present. The condition was somewhat poor. The abdomen sunken. Integument: bullet wound in the head. Swollen lips, abrasions on frontal regions. The eyes were closed. Decubitus on hips. The anus was closed. The visible mucous membranes of the eyes were pale, with a yellow tinge. The blood was not coagulated, it did not stain very well and had a brownish tinge. No fat was in the subcutaneous tissue. The aponeuroses and fasciae were pale yellowish. The muscles when cut appeared somewhat dry. The submaxillary gland showed nothing unusual, neither did the guttural pouches; in the postpharyngeal tissue was a large haemorrhage (bullet). The mandibular lymph glands were moist, the retro-pharyngeals on the left side were embedded in a blood coagulum (bullet). The upper cervicals were moist. The thyroids were somewhat pale, normal in size. At the base of the tongue was some thick mucous deposit. Palatinum and pharynx showed nothing unusual. The larynx contained a little mucus. The mucosa of the trachea was pale, the veins were injected. No lesions on the tongue. The intima of the aorta smooth and glistening. The peritoneal serosa was smooth, moist, and glistening. The diaphragm was convexly forwards. Fibrous filaments were present on caudal surface, the oral one was smooth. The anterior mediastinal lymph glands were oedematous, also the lower cervicals, the supporting tissue was gelatinous. No foreign contents were present in the pleural cavities. The costal pleura was smooth and glistening. The bronchial lymph glands were slightly swollen and black, the tissue was gelatinous, oedematously infiltrated. The apex of the right lung was adherent to costal pleura by fibrous filaments. After breaking them there were extensive fibrous adhesions on the fourth rib. Left lung: emphysema in apex, also on lower border of main lobe, the surface was uneven, there was contraction of pleura which was white and thickened. On section gelatinous infiltration in tissue was fairly extensive. The posterior border of the cardiac notch was hard, and on section had a gelatinous fleshy appearance. Serum was escaping. The pleura of the diaphragmatic surface was white and thickened, and contained many fibrous filaments. On section the parenchyma of the basal lobe was elastic and contained a fair amount of blood. The main bronchus contained a little froth. The intima of vein and artery were smooth and glistening. Right lung: emphysema and consolidation were noted in apex; on section an empty cavern was noted lined by a red membrane. The anterior portion of main lobe was somewhat hard to the touch. On section it appeared oedematous. On section of the main lobe a considerable amount of blood escaped. The parenchyma was elastic. The intima of the vein showed nothing unusual. Little froth was in the bronchus, the mucosa was normal. The artery contained partly coagulated blood. No traces of fat under the pleura of the pericardium. The pericardium contained 20 c.c. yellow liquid. The parietal serosa was smooth and glistening. A few vessels were injected. Both ventricles and atrium were empty. The epicardium was smooth and glistening, a few ecchymoses were noted at the base. A fair amount of fat in the grooves. The left endocardium was smooth and glistening. Few brownish ecchymoses were in the septum and also a yellowish infiltration. A similar infiltration was noted in the bicuspid valve. The right endocardium showed ecchymoses in the papillary muscles and in tricuspid valve, the valves were yellowish. The endocardium was smooth and slightly pale. Yellowish infiltration was noted in the annulus circularis. The left wall was 3.8 and right 2 cm. thick. The intima of the coronary arteries was smooth and glistening. Myocardium of septum was pale grey opaque, the consistence was about normal. The periportal lymph glands were oedematous and gelatinous. The liver was fairly rich in blood, the size was normal. The edges were fairly sharp. The capsule was smooth. Calcareous nodules were present, size of a pea. On section the parenchyma had a greenish tinge and a peculiar tracing of light brown and yellow meshes. A lobulation was not discernible. The consistence was somewhat firm. The pancreas was fairly rich in blood, it was dark brown in colour and somewhat hard. Sclerostomum larvae were found within. The splenic lymph glands showed nothing unusual. The spleen was somewhat small, 36 × 19 cm., the capsule was shrivelled, a few black petechiae were present. The pulp was dark in colour, the trabeculae were visible, the follicles were not distinct, the consistence normal. The right suprarenal gland was rather dark in colour. The cortex showed yellow striations and the left suprarenal glands were similar. Kidney: the capsule contained a small amount of fat. The right capsule was easily detached, the organ had yellow mottled appearance on surface. The cortex was slightly yellowish, in the medulla were some haemorrhages, surrounded by yellow areas. The left capsule was also easily detached. The surface of the organ was mottled brown yellow in colour. On

section the cortex was pale, light brown, moist, transparent, with yellow blotches, in substance corresponding to yellowish areas on surface. Stomach: some food and liquid present. The mucosa of the fundus showed nothing unusual. No parasites were present. A large bunch of gastrophilus larvae were in the duodenum. The mucosa was greyish coloured. The mucosa of the ileum and jejunum was slightly swollen. The floating colon contained mucous deposit. The caecum and colon mucosa showed greyish discoloration. Mesenteric lymph glands: the supporting tissue was oedematous. Intima of the abdominal aorta was smooth and glistening. The intima of the arteria ileo-caeco-colica was somewhat thickened. The bladder was half full of yellow somewhat turbid urine, the mucosa showed nothing unusual. The mucosa of the rectum was somewhat pale. Thrombi were present in vena pudenda externa. The brain was haemorrhagic, moist, rich in blood, small cholesteatoma was found in right ventricle. Skeleton: depression in the last three molars. The marrow in the femur was fatty with a haemorrhagic focus. The humerus contained fat. Body weight: 322.3 kg., right lung 3.6 kg., left lung 2.5 kg., and left kidney .8 kg. Liver weight: body weight = 1 : 67.

Pathological anatomical diagnosis.—Decubitus. Icterus. Chronic interstitial pneumonia; bronchi-ectasis; infarcts and fatty degeneration of kidneys; atrophy, fatty degeneration and pigmentation of liver; parasites in stomach, pancreas, and intestines.

Microscopical examination.—The lobulation could be recognized. In a lobule three zones could be distinguished; around the central vein, embracing about half of the lobule, was a zone of liver cells. These were not intact, they showed fine granular brownish pigment, some showed vacuoles, some were faded, the cytoplasm was absent, and the cell body had a greyish transparent appearance. The nuclei showed various degrees of fading, some were just discernible by their outlines and the presence of a nucleolus (Karyolysis). In the peripheral portion of this central zone the capillaries were injected and formed a more or less defined zone around the centre, the liver cells enclosed by the capillaries showed generally the same characters as described, there were more vacuoles and larger ones present. In some places the cells were absent, and blood corpuscles occupied the place and the pigment seemed to be loose between them. The peripheral zone consisted of what must be described as young bile-ducts. They occupied almost the whole periphery of the lobule and formed broad bands, taking up about one-quarter of the lobular diameter, contrasting distinctly by their pale grey colour from the liver cells. They enclosed still some of the latter, pushing between them and so that only remnants of liver cell rows or liver cells were left. In some places the bile-ducts were cut cross-ways and showed here a small lumen, the cells were radially placed and wedge-shaped, in some places they were longitudinally cut and again a narrow lumen was visible; these bile-ducts entered in some places fairly deep into the periphery of the lobule. In some places no lumen could be made out, but two distinct rows of cells, well defined, rectangular in outlines, were running parallel; they apparently represented newly formed liver cell rows. Left lobe: the injection of the blood-vessels was absent. The lobules had a patchy appearance due to the absence of cytoplasm in liver cells of certain portions of the lobules; these portions appeared greyish transparent. On closer examination liver cells could not always be made out; in their place was a finely areolated substance, foam like and confluent. Portions of the periphery showed similar conditions. In some cells large vacuoles were present. The pigment seemed to be free. There was a small round cellular infiltration in the septa, particularly in the walls of the blood-vessels. In the Sudan-stained section the presence of much fat was shown; in the outer portions of the lobule the cells were crammed full with fat globules, also some of the newly formed bile-ducts showed the fat globules in a moderate degree; fine fat globules were present in the epithelial cells of the larger bile-ducts. Lobus quadratus: in some parts the newly formed bile-ducts occupied a considerable portion of the lobule (almost half of it), and the capillaries showed well up between them. Pigment was present in the interstitium. Individual or clusters of old liver cells were still present. Pigment was also present in the cells of the septa. In the Sudan-stained section extensive fatty degeneration was noted, in particular in the outer portions of the lobules, where fat globules were coalescing forming large drops. In the cells of the newly formed bile-ducts were also extensive fat agglomerations. There were parasitic nodules present. Kidney: in the Sudan-stained section practically all the tubuli contorti and recti showed fat reaction in the form of small globules cramping the epithelial cells. The glomeruli were free of it. Also the larger tubuli

uriniferi showed none or only little, whilst the tubules of Henle showed most. In the Haemotoxylene-eosine section in a number of tubules no nuclei were present, in the majority the nuclei showed a deep dark stain. This applied to Henle's tubules. In the cells of the tubuli uniferi a peculiarity was noted, viz., some of the cells showed a white halo, mostly around the nucleus, whilst the margin stained deeper. These cells, therefore, appeared very light coloured, and contrasted to the deeper colour of the tubules of Henle. There was a patch of localized hyperaemia in this section; the glomeruli belonging to it as well as all the capillaries were fully engorged, and most of the tubules enclosed by it were devoid of nuclei or showed pyknotic nuclei. In the glomeruli serum was seen between wall and tuft. In a number of tubuli contorti homogeneous pinkish-stained substance was present, in some it was retracting from the walls. Heart: the Sudan stain showed already macroscopically a diffuse staining of the section, and microscopically the fibres were crowded with globules of considerable size, so that the structure in parts was obscured. There were also fine fat granules present in Purkinje's fibres. Skeleton muscle: some of the fibres showed distinct fat globules; usually a fibre in its whole length was so altered, rarely a few at a time. Suprarenal glands: already macroscopical inspection showed the cortex diffusely red by the Scharlach stain and microscopically the cortex showed up to be intensively stained, in some places obscuring the nuclei. Also in the medulla were patches of fat, obscuring the cells in which they were lodged. Lungs: the bronchi showed wide lumens on cross section and within a plug of pus cells. The alveoli in the vicinity were filled with polymorphonuclear cells in such a way that their lumen had a uniform aspect. The alveolar septa were quite distinct, the capillaries filled with red corpuscles. At one place, where the largest collection of polymorphonuclears was visible, a necrotic patch was present in the centre around a lumen without any wall structure. Here septa could still be recognized, the alveolar contents, however, consisted of homogeneous brown substance; in the periphery a very thin zone of nuclei was undergoing karyorrhexis, and black patches of apparently dissolved nuclear substance were present (wall of demarcation). Amongst the polymorphonuclears were also some degenerated desquamated cells. In certain portions of the section the alveoli were filled with homogeneous substance (serum), with serum and desquamated cells or these two and polymorphonuclears.

Diagnosis.—Necrosis and fatty degeneration of liver cells, pigment, and regeneration of bile-ducts. Fatty degeneration of kidney and necrosis of the tubuli recti. Fatty degeneration of the suprarenal glands. Broncho-pneumonia.

CASES OBSERVED AMONGST DEFENCE FORCE HORSES THAT WERE NOT IMMUNIZED.

- (1) Horse B183, a bay gelding, admitted to the Veterinary Hospital, Roberts Heights, on the 19th March, 1917. It died the same day. This horse was not immunized.

Microscopical examination.—Liver: the staining of this section was not good, post-mortem changes preventing it. Nevertheless, the picture of acute liver degeneration could be made out. A homogeneous detritus in the central portion of the lobule and tubes of proliferating liver cells in the periphery. In the Sudan-stained section it could be recognized that almost the whole lobule had undergone fatty degeneration, large fat droplets being present more frequently arranged in the peripheral than in the central portions. Some of the large fat globules showed also vacuoles.

Diagnosis.—Necrosis and fatty degeneration of the liver cells. Regeneration of bile-ducts.

- (2) Horse K2437, admitted to the Veterinary Hospital at Roberts Heights on the 2nd April, 1917; it died on the 3rd April, 1917.

Post-mortem.—Liver slightly smaller than normal and apparently in state of fatty degeneration. The spleen smaller than normal, the other organs appeared normal.

Microscopical examination.—The Sudan stain showed an extensive fatty degeneration of the liver cells principally around the central veins, the fat globules being of a large size. The remainder of the lobule was occupied by homogeneous detritus, in which nuclei and round cells could be recognized. In the Haemotoxylene-eosine stained section, detritus could be recognized in

the centre of the lobule. The septa in many parts showed considerable accumulation of round cells. Some of the cells in the periphery belonged to budding bile-duct cells; the staining owing to post-mortem changes was somewhat indifferent. There was an accumulation of blood in some lobules. In another section the budding liver cells could be seen better, they also showed vacuoles. Kidney: extensive fatty degeneration present in all tubuli contorti and in many tubuli recti.

Diagnosis.—Fatty degeneration and necrosis of liver cells, emigration of round cells. Fatty degeneration of kidney.

(3) Horse L771, a seven-year-old black gelding, admitted to the sick-lines at Durban on the 27th April, 1917, showing symptoms of Staggers (diagnosed as Dunziekte). No treatment was of any avail to give even temporary relief. This horse was purchased at Vrede by "L" Purchasing Board on the 4th June, 1916. It was sent direct from Vrede to the Durban Remount Depot, and had been kept under ordinary conditions. This horse was not immunized. It died on the 28th April, 1917.

Post-mortem.—Condition good. Lungs congested and oedematous. All subcutaneous tissue jaundiced. Acute gastritis, stomach distended with water, no food present. Liver acutely congested. Kidneys were congested. Urine saffron coloured. Haemorrhages in both ventricles of the heart. From the symptoms and the intra vitam post-mortem appearance the cause of death was diagnosed as "Acute Dunziekte."

Microscopical examinations.—The borders of the lobules stood out distinctly, forming an irregular meshwork, and the areas bordered by the meshes were sunken. The gross lobular arrangement was still present in its outlines, the finer structure was, however, absent. There were no liver cells left. Around the central vein and occupying the greatest portion of a lobule the liver cells were replaced by blood; here still some radiating structure could be made out in some parts, the capillaries being simply distended and occupying the place of the absent liver cell rows, and indicated by the position and direction of cells with spindle-shaped nuclei which were the endothelial cells of the capillaries. There were also a number of round cells present in the blood, but their number seemed to attain normal proportions. In some places amongst the red cells were vacuoles left; around the central vein in particular they could be recognized as vacuolated liver cells (fat cells); in a Sudan section such vacuoles distinctly showing the fat drops. In the periphery was a zone of newly formed liver cells; large cells, with numerous vesicular nuclei and of the colour of bile-ducts, were arranged with their longitudinal axis centrally directed. Sometimes these cell tubes were fairly long and straight, or convoluted and running then in the direction of the septum. On cross section they showed the picture of a bile-duct, a central lumen, and radially arranged cells. Some of these showed vacuoles, and in the Sudan section these vacuoles showed up as fat droplets. In the septa were round cells, usually scattered, but sometimes forming clusters. The septa in parts were distended by homogeneous substance (serum).

Diagnosis.—Fatty degeneration and necrosis of the liver cells, emigration of round cells; regeneration of bile-ducts and liver cells.

APPENDIX II.

CASES OF EXPERIMENTAL *Senecio* POISONING.

I. FEEDING EXPERIMENTS WITH *Senecio latifolius* IN NATAL.

For this purpose Mooi River was selected, a locality renowned for Dunziekte and where the Ragwort weed was much in evidence. The Government Botanist made a survey of the Dunziekte area and pointed out various species of *Senecio*, which were at that time suspected to be the cause of this disease, the most common one being *Senecio latifolius*, and it was decided to start with the feeding of this plant. The Government Veterinary Officer of the district was placed in charge of the experiment, for which a number of horses were selected. The notes of these cases are taken from his report.

Feeding Experiments with Green Plants in Mooi River.

Experiment No. 1.—Horse 5122 arrived in Onderstepoort in April, 1910. It was immunized against Horse-sickness and subsequently hyperimmunized. On the 24th October, 1911, it was sent to Mooi River. The feeding was commenced on the 1st November, 1911. The green plant was mixed with bran and mealies after it had been passed through a mincing machine. The horse ate 1 lb. daily for the first six days; on the 7th, $1\frac{1}{2}$ lb.; on the 8th, 2 lb.; 9th, 2 lb.; 10th, 11th, 12th, 3 lb.; 13th, 4 lb.; 14th, $4\frac{1}{2}$ lb.; 15th, 16th, 17th, and 18th, 3 lb. daily; 19th, 2 lb.; 20th, 2 lb.; 22nd, 23rd, 24th, 25th, and 26th, $\frac{3}{4}$ lb. daily; 27th, $1\frac{3}{4}$ lb.; 28th, 2 lb.; 29th, $2\frac{1}{4}$ lb.; 30th, 3 lb.; 1st December, 3 lb.; 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th, and 10th, 2 lb. daily; 11th and 12th, 3 lb. daily; 13th, 14th, 15th, and 16th, 2 lb. daily; 17th and 18th, 1 lb. daily; 19th, $\frac{1}{2}$ lb.; 20th, 21st, 22nd, 23rd, 24th, 25th, 26th, and 27th, $\frac{1}{4}$ lb. daily. After this date the horse refused to eat any food containing the plant. The plant was then made into a ball of 5 oz. on the 28th, and afterwards $\frac{1}{2}$ lb. daily was given constantly in the form of balls. The total amount consumed up to death was 104 lb. 5 oz. in eighty-four days. During the first month the horse improved, and during the last fortnight it lost in condition. Up to the 17th November, 1911, the horse's faeces were soft, but after that date they became formed and pasty in appearance. About a week before death the dung was rather hard, dry, and reddish brown in colour. On and after the 17th the conjunctiva became injected and showed blood spots on the membrana nictitans. Two days before death the conjunctiva had a dirty yellow colour with a few bright red spots. On the 10th January, 1912, the urine was noted to be of a brownish red colour, somewhat the colour of coffee, and this continued up to the time of death. The temperature remained normal throughout the experiment. For the last fortnight the pulse had been very distinct and quick, about 60 per minute; on the evening of the 15th January, 1912, the horse refused food altogether. Signs of uneasiness were shown by striking with its hind legs towards the stomach; it was lying down occasionally for short periods but not rolling. The pain did not appear to be caused by intestinal troubles, but was apparently attributable to some disturbance in the kidneys, as the horse after each period of pain passed a small quantity of coffee-coloured urine. Whilst standing it would sway backwards and forwards, elevate its head, and depress its hindquarters. There was no straining. The pulse was 100 to the minute and thready. There were not any signs of brain trouble and the horse remained perfectly sensible up to the time of death. There was no boring of the head into the manger or other objects as is always seen in cases of "Dunziekte." Its gait, although unsteady during the last twenty-four hours, was not the gait of a "Dunziekte" horse. A few hours before death the weakness across the loins became more and more evident, and the horse each time it lay down had increasing difficulty in raising its hindquarters. On the 16th January, 1912, its pulse was 120 per minute, the heart could be seen beating against the chest wall; the artery was very thready, temperature 100.4° F., the extremities were warm and remained so until death, the previous evening they had been cold. Salivation was seen and a grey mucous discharge from both eyes; respiration until twenty-four hours before death had been normal. There was a tumefaction of the skin about the chin which may have been caused by an injury, although no abrasion could be detected. The horse died at noon and a post-mortem examination was made immediately afterwards.

Post-mortem.—The fat throughout the body was of a yellow colour. The lungs were apparently normal but very pale. The heart was normal, except that the muscular tissue felt exceptionally firm. Liver considerably enlarged, edges rounded, colour of surface bluish grey, consistence abnormally firm. Nutmeg appearance on section throughout, probably cirrhotic. Spleen was enlarged, edges rounded, outer surface showed intense congestion. Stomach contained fair quantity of sour-smelling food; cuticular portion was normal, a few bots were present. The villous portion, when washed, had what appeared to be green herb juice firmly attached to surface, this could be scraped off and the mucous membrane appeared normal. Intestines: the duodenum contained a yellow mucus-like substance; the other small intestines contained a large quantity of thick material of a dark reddish brown colour, almost the colour and consistence of tar. After it was washed off the mucous membrane appeared normal. The caecum was normal. The mucous membrane of large colon showed throughout patchy infiltration. Floating colon and rectum contained hard dry faeces together with a quantity of yellow stringy mucus. Kidneys: both were much enlarged, the surface showed areoles studded with black spots; cut surface showed

intense congestion with black streaks here and there running through the cortex. Urinary bladder contained dark yellowish brown urine.

Pathological anatomical diagnosis.—Anaemia. Icterus. Stasis of liver and fatty degeneration. Hepatitis. Tumor splenis. Nephritis haemorrhagica. Haemoglobinuria. Enteritis catarrhalis. Gastrophilus larvae in stomach.

Result.—This horse received in eighty-four days a total amount of 104 lb. 5 oz. of Ragwort. It died of Ragwort poisoning. Although a microscopical examination of the liver was missed, the naked description of it can be interpreted as that of a parenchymatous Hepatitis. The haemoglobinuria was well pronounced, and some of the symptoms seen during life apparently find their explanation to be due to the disturbance in the functions of the kidney. In this case therefore lesions in liver and kidney were present. The tachycardia noted ante-mortem points also to heart lesions.

Experiment No. 2.—Grazing of a horse in a paddock with an abundance of Ragwort to see whether it would feed of it by its own choice. Horse 5772 arrived at Onderstepoort in January, 1911. It was immunized and hyperimmunized against Horse-sickness. On the 24th October, 1911, it was sent to Mooi River. The horse was turned out into the paddock on the 1st November, 1911, since which date it had been running in the paddock and grazing daily on the pasture. It was, however, never noticed that it ever touched any of the Senecios. They were left alone, and when all the grass had been eaten down, they still stood there. On the 4th December, 1911, the horse appeared well and was looking better than when it arrived.

Result.—This was an experiment to see whether horses will eat Ragwort of their own choice, if they have access to it. The horse did not touch the plant. This experience is a common one made by the farmers, who most emphatically state that they have never seen horses eating the weed, or if so, only accidentally.

Experiment No. 3.—Horse 5779 arrived at Onderstepoort in January, 1911. It was immunized and hyperimmunized against Horse-sickness. It was sent to Mooi River on the 24th October, 1911. On the 1st November, 1911, it was put in the Ragwort feeding experiment. Up to the 31st December, 1911, this horse had received $\frac{1}{4}$ lb. daily, minced and mixed with bran and mealies. No trouble was experienced to get him to eat this amount. The horse appeared to be in good health. Total amount consumed up to the 31st December amounted to $15\frac{1}{4}$ lb. in sixty-one days.

Result.—The horse consumed in sixty-one days a total amount of $15\frac{1}{4}$ lb. Ragwort. No symptoms of poisoning were noted during this period.

Experiment No. 4.—Horse 5402 arrived at Onderstepoort on the 21st February, 1910. It was immunized against Horse-sickness. On the 3rd January, 1912, it was sent to Mooi River. It received daily 6 oz. of *Senecio latifolius* or a total amount of $8\frac{1}{2}$ lb. The experiment had to be discontinued owing to scarcity of plants.

Experiment No. 5.—Horse 6152 arrived in Onderstepoort in May, 1911. It was immunized against Horse-sickness. On the 3rd January, 1912, it was sent to Mooi River. It received daily 6 oz. of *Senecio latifolius* or a total amount of $8\frac{1}{2}$ lb. The experiment was discontinued owing to the scarcity of plants.

Experiment No. 6.—Horse 4259 arrived in Onderstepoort in May, 1909. It was immunized and hyperimmunized against Horse-sickness. On the 3rd January, 1912, it was sent to Mooi River, and received daily 6 oz. of *Senecio latifolius* or a total amount of $8\frac{1}{2}$ lb. The experiment was discontinued owing to the scarcity of plants.

Result of Experiments Nos. 4, 5, and 6.—Each horse consumed a total amount of $8\frac{1}{2}$ lb. Ragwort. No symptoms were noted during this period or thereafter.

II. FEEDING OF DRIED SENECIO SPECIES IN ONDERSTEEPOORT.

The material for this experiment was obtained from the Government Veterinary Officer in Kokstad. There were several species forwarded, viz.: *Senecio latifolius* (D.C.), var. *barbellatus* (D.C.); *S. adnatus* (D.C.); *S. gramineus* (Harv.); *S. serra* (Sond.); *S. albanensis* (D.C.), var. *angustifolius* (Harv.); *S. coronatus* (Harv.); *S. bupleuroides* (D.C.). The majority belonged to the species *S. latifolius* (D.C.), *adnatus* (D.C.), and *albanensis* (D.C.). The feeding was mainly with *Senecio latifolius*.

Experiment No. 7.—Horse 8220, an aged dark brown gelding obtained from the Principal Veterinary Officer, Army, Potchefstroom, on the 24th June, 1913.

Anamnesis.—It passed the mallein test on the 25th June, 1913, with negative results. On the 27th September, 1913, it was submitted to the Ragwort feeding experiment, receiving 1 lb. of Ragwort from the 27th September to the 12th November. The temperature during this period kept fairly regular, except for some fever exacerbations on the 4th October, rising to 102° F. In the evening of the 19th October the thermometer registered 102.4° F. and on the 26th 102° F. From the 21st November, 1913, to the 3rd December, 1913, the horse received daily 3 lb. of Ragwort, and also on the 8th and 9th and from the 18th to the 23rd. The temperature kept normal during the whole period. From the 30th December, 1913, to the 2nd January, 1914, and from the 7th to the 9th, it received 2 lb. daily, the temperature still keeping normal. From the 13th to the 15th it again received 2 lb. daily. This time some fever exacerbations, rising on the 14th to 104.2° F., were noted. The horse consumed 130 lb. in seventy-eight days. On the 17th the horse was submitted to a Horse-sickness experiment, receiving intrajugularly a first injection of 3 c.c. virus of horse 7284, Tzanec, 13th generation. On the 23rd it received the second injection intrajugularly, viz.: 100 c.c. serum "O" 1702 and 3 c.c. virus of horse 8368, Webster, 2nd generation. On the 28th a third injection was given also intrajugularly of 100 c.c. serum "O" 1702 and subcutaneously 2 c.c. virus of horse 8408, Bulawayo, 13th generation. There was a slight reaction lasting from the fourth to the fifteenth day after the first injection. The temperature was somewhat irregular, rising to 102.8° F. in the evening of the 23rd. The minimum, 99.6° F., was observed in the morning of the 26th. On the 31st January, 1914, on inspection abrasions in the skin of the supra-orbital process and of the forehead were noticed. The horse was found pushing the head into a corner with the forelegs spread out. On the 1st and 2nd February a staggering gait was present, the hind legs were dragging. The animal refused to feed and was semi-comatose. It soon mended, however, and recovered.

Result.—This horse received a total amount of 130 lb. Ragwort in seventy-eight days without showing any symptoms during this period. The feeding was then discontinued and the horse was put into a Horse-sickness experiment. Subsequently at about the conclusion of the Horse-sickness reaction, which was very mild, the horse developed symptoms of Staggers in a mild degree, from which it recovered. At the time the symptoms were not exactly understood, seeing that the feeding of the poisonous weed had shortly before been discontinued. In the light of subsequent events, however, they found an explanation.

Experiment No. 8.—Horse 8225 arrived at Onderstepoort on the 24th June, 1913, from the Principal Veterinary Officer, Army, Potchefstroom. On the 25th June, 1913, it passed the mallein test with negative result. On the 27th September, 1913, it was submitted to a Ragwort feeding experiment, receiving daily 1 lb. until the 12th November, 1913. The temperature during this period kept fairly normal, only on one occasion a fever exacerbation to 102.2° F. was noted. From the 21st November, 1913, to the 3rd December, 1913, the horse received 3 lb. of the plant daily. The feeding was then discontinued for a few days. On the 9th and 10th the horse again received 3 lb., and the same quantity from the 18th to the 23rd. From the 30th December to the 2nd January, and from the 7th to the 10th, 2 lb. were fed daily. During the evening of the 10th a fever exacerbation to 103° F. was noted. The feeding was discontinued for two days. The horse again received 2 lb. for the next three days. A total amount of 132 lb. was consumed in seventy-nine days. On the 17th January, 1914, the horse was submitted to a Horse-sickness experiment and received an intrajugular injection of 3 c.c. virus of horse 7284, Tzanec, 13th generation; on the 23rd 100 c.c. serum "T" 1703 were given and subcutaneously 10 c.c. virus of horse 8368, Webster, 2nd generation. On the 28th 100 c.c. serum "T" 1703 were injected into the jugular vein. The temperature kept normal until the 23rd when the typical Horse-sickness reaction commenced. It rose to 104.2° F. on the 27th. The horse died during the night of the 28th. The conjunctiva on the day of death was noted to have a yellow tinge and ecchymoses.

Post-mortem report.—The autopsy was made eight hours after death. The condition was fair, the abdomen not distended, and rigor mortis was present. Abrasions on the head were noted. The mouth had a bluish tinge, the flesh a brownish colour, the subcutaneous tissue a brown yellowish tinge, and was moist. The muscles were slightly brown tinged and also moist. The blood was partly

coagulated, it appeared black. The diaphragm on posterior side showed numerous fibrous filaments and on the anterior side a few fibrous thickenings. The mucosa of the pharynx was diffusely reddened. The connective tissue around the pharynx showed some oedematous infiltrations. The epiglottis and larynx were diffusely reddened and injected. The follicles at the base of the tongue were distinct. The upper end of the oesophagus contained some food. Submaxillary glands were darker than normal, also the parotid glands. Nothing particular was noted in submandibular lymph glands. The upper cervical lymph glands were somewhat enlarged and congested. The lower cervical lymph glands were enlarged and embedded in an oedematous tissue. The bronchial and mediastinal lymph glands showed haemoglobin infiltration. The nasal cavities were normal. The pleural cavities contained a small quantity of dark reddish brown fluid. The peritoneal cavities contained about 130 c.c. brown yellow stained liquid. The left wall of the peritoneum showed a cicatrix, 14×2 cms. The trachea was reddish. The pleura of right anterior lobe showed gelatinous infiltration, pleura of left lung was whitish. Consistence of lung was spongy, on section it appeared not so rich in blood as the right lung. A plasma coagulum of dirty brown colour was found in the vessels. The walls of the pericardium were rich in fat. Left endocardium was diffusely haemoglobin stained, particularly in musculi papillares. Extravasations of blood were noted under the endocardium. The myocardium was dark in colour, it was soft. Epicardium showed a brown reddish tinge, which was more pronounced in the fat. Both ventricles contained some coagulated blood. The intima of the coronary artery was smooth. The aorta thoracica was slightly red brown tinged. Liver: anterior side of right lobe showed fibrous filaments. It was green in colour. Glisson's capsule was smooth. Ductus choledochus was open. Consistence of liver tissue was normal. On section it was light yellow brown, rich in blood. The lobulation was distinct. In the periportal lymph glands was some calcareous deposit. Spleen was slightly enlarged, 41×28 cms. Its lymph glands were slightly enlarged and moist. The pulp was dark in colour. Numerous calcareous nodules were present. The capsule tense and showed fibrous filaments. Stomach contained food, the mucosa of the fundus was bluish red, the pylorus showed hyperaemic streaks. Mucosa of small intestines, caecum, and colon were red in colour and slightly swollen, the mucosa of the rectum was slightly slate coloured. The mesenteric lymph glands were slightly enlarged and oedematous. Kidneys: left was very dark, intermediary zone and cortex were confluent. Right kidney was larger than left. The glomeruli were distinct. The pelvis was haemoglobin stained, it contained a small coagulum of soft consistence. Suprarenal glands were dark in colour. The urinary bladder contained haemoglobin stained urine and red corpuscles. The urethra was also diffusely reddened. The vagina contained a brown red discharge. The udder was normal. Left ovary was enlarged, fibrous, containing some cysts and a fresh corpus luteum. The right ovary was smaller and also showed cysts and numerous corpora alba. Brain: the pia was injected, the brain substance somewhat dark in colour. At the right entrance of the nervus opticus was a small haemoglobin stained infiltration. The femur showed red marrow along the tela ossea and a diffuse blood infiltration of 6×2 cms. in the centre of the marrow. The humerus was normal.

Pathological anatomical diagnosis.—Icterus. Traumatism on head. Oedema of connective tissue of the neck and the pharyngeal lymph glands. Dissolutio sanguinis. Slight hydrothorax and ascites; petechiae and ecchymoses in left endocardium. Stasis and fatty degeneration of the liver. Slight tumor splenis, hyperaemia of the mucosa of the stomach and the intestines. Calcified parasitic nodules in spleen. Hyperaemia and cloudy swellings of the kidneys; haemoglobinuria and haematuria. Calcareous degeneration of portal lymph glands. Cystic degeneration of left ovary. Haemorrhage in nervus opticus. Hyperaemia of the brain.

A microscopical examination of the liver and kidneys was unfortunately missed.

Result.—The horse received a total amount of 132 lb. Ragwort in seventy-nine days. The feeding was then discontinued and the horse was placed in a Horse-sickness experiment. It developed a typical Horse-sickness reaction and died without giving much warning. The autopsy revealed the presence of lesions of Horse-sickness and of Senecio poisoning; the former obscuring to some extent the latter. The lesions of Senecio poisoning were rather strongly pronounced in the kidneys and both haemoglobinuria and haematuria were present.

Also in this case the disease developed after the feeding had been discontinued, and it was uncertain at the time whether it was due to the intercurrence of Horse-sickness. Since both the horses (Experiments Nos. 7 and 8) developed the disease at about the same time, such a possibility cannot be excluded. In the light of subsequent events it may also be considered to be a coincidence.

Experiment No. 9.—Horse 9451, an aged grey gelding, which arrived at Onderstepoort on the 10th August, 1915. It passed the immunization of Horse-sickness with the symptoms of Dikkop. It was subsequently tested with "O" virus of the 175th generation and proved immune to Horse-sickness. On the 11th November, 1915, it was put into a Ragwort experiment, receiving from the 11th to the 15th daily 1 lb. of *Senecio latifolius* mixed with oats. From the 16th to the 19th, 2 lb. Ragwort mixed with oats and crushed mealies, from that date daily to the 1st December, 2½ lb. were given. The animal never fed well and on several occasions refused to eat the plant. It was therefore on the 3rd December dosed with the plant made into a ball. After this the feeding was discontinued until the 8th December. From the 9th onwards quantities of 1 lb. and ½ lb. and 2 oz. were supplied and eaten. A total amount of 43 lb. 4 oz. was consumed in forty-four days. During the period of feeding the temperature kept fairly regular, with the exception of an occasional exacerbation, which was only once above 101° F. The animal, however, noticeably lost condition from the 19th November onwards. It showed a tucked-up appearance; this loss of condition increased rapidly as time proceeded. The horse died during the morning of the 13th December.

Post-mortem report.—The autopsy was made two hours after death. Condition was very poor. Rigor mortis was not present. The integument showed decubitus on hips, elbows, and shoulders. The conjunctiva was slightly reddish yellow. The flesh was somewhat dark. Little fat was present in the subcutaneous tissue. The costal pleura was reddish coloured. Diaphragm convexly forwards. Few fibrous filaments were present on both sides. The serosa of peritoneal cavity was slightly reddish yellow. Bronchial and mediastinal lymph glands were moist and slightly enlarged. The retropharyngeals slightly pale and moist, also the submaxillaries. Thyroids were normal. Tongue, pharynx, and oesophagus were normal. Respiratory organs: right lung was not collapsed, emphysema was present in anterior and on border of main lobe; the pleura was slightly yellowish; on section colour and consistence of lungs were normal. Mucosa of bronchus was yellowish in colour. The left lung was normal. Mucosa of trachea was slightly reddish yellow. Pericardium contained 20 c.c. reddish liquid. Circulatory organs: blood was coagulated in both ventricles. Epicardium was slightly dark, base gelatinous, in left transverse sulcus was a haemorrhage. Right endocardium showed marked haemorrhages on musculi papillares and blood-streaks on walls, otherwise it was yellowish tinged. In left endocardium were also ecchymoses. Myocardium was pale yellowish. Intima of coronary arteries was normal. Intima of pulmonary artery was yellowish. Intima of thoracic and abdominal aorta was also slightly yellowish. Anterior mesenteric artery showed a thrombus. Liver: fibrous patches and filaments were present on anterior surface. One calcareous nodule was found in right lobe. Liver lobules were very distinct, the periphery was greyish and slightly raised. Consistence was firm. Pancreas was normal. Spleen: 43 × 26 cms. The capsule was yellowish. The pulp on section was dark red and slightly firm in consistence, trabeculae were distinct, the follicles were visible. Suprarenal glands were normal. Kidneys: capsule was poor in fat, fibrous capsule was slightly adherent. On section the zones were distinct, the medulla was slightly hyperaemic. The cortex was brownish green with reddish spots. Stomach: gastrophilus larvae were frequent. Mucosa of fundus showed diffuse hyperaemia. Small intestines: mucosa of duodenum was swollen and showed a slight diffuse hyperaemia, that of the jejunum was swollen and hyperaemic, in some places well marked. Ingesta were mixed with blood. Large intestines: patches of hyperaemia were present. Small colon showed a slight diffuse hyperaemia of the mucosa. In rectum balls of faeces were present. The mesentery was normal. The bladder was much distended with dark chocolate-coloured turbid urine, mucosa showed a diffuse hyperaemia and numerous ecchymoses. The vessels were injected. The sexual organs were normal. Nervous system: brain was normal. Skeleton: bone marrow was gelatinous, both in humerus and femur.

Pathological anatomical diagnosis.—Traumatism. Slight cachexia. Icterus. Dissolutio sanguinis. Cloudy degeneration of myocardium; haemorrhages in endocardiums and epicardium. Hyperaemia of kidneys. Parenchymatous

degeneration of liver; perihepatitis fibrosa, calcified parasitic nodule in liver. Slight haemorrhagic gastritis; haemorrhagic enteritis. Haemoglobinuria. Ecchymoses and hyperaemia in bladder. Thrombosis of mesenteric artery. *Gastrophilus* larvae.

Microscopical examination.—The walls of the central veins were thickened and hyaline. The lumen was filled with red corpuscles, also the immediate circumference, obscuring everything, so that no liver cells were seen. There were clusters of pigment along the wall of the central vein. Around this haemorrhagic centre was a zone of pale coloured cells, all with numerous nuclei of fairly large size, apparently budding bile-ducts and new liver cells. Between them were left some old liver cells. In the periphery of the lobule they were irregularly shaped, angular, with deep nuclear staining; they were somewhat rare. The septa were not much thickened, here the bile-ducts were distinctly tubular and had increased in numbers. There were also round cell collections in the centres and in the septa. Kidney: the blood-vessels contained a fair number of red corpuscles. The glomerulus capsule contained a faintly stained substance which had the shape of globules of various size. The smallest were larger than a red corpuscle, the largest were $12\ \mu$ in diameter; the tuft was pushed to the opposite pole of the capsule, so that this substance occupied a semi-lunar space; some similar substance was also found in some of the tubuli contorti; some of the tubuli also contained red corpuscles, some a homogeneous faintly stained and some a rust-red substance. Generally the parenchyma did not stain well. Quite a number of cells in the tubuli contorti showed fat. Heart: under the endocardium was a fairly extensive haemorrhagic infiltration. The heart muscle was patchy with fatty degeneration; the patches were irregularly distributed; the degeneration was sometimes more pronounced in the fibres belonging to the periphery of a muscular bundle.

Pathological anatomical diagnosis.—Hyaline degeneration of the central veins. Stasis of the liver, necrosis and atrophy of liver cells; pigmentation; emigration of round cells; regeneration of bile-ducts. Cloudy and fatty degeneration of the kidney; haemoglobinuria. Fatty degeneration of the heart.

Result.—This horse received over a period of forty-four days a total amount of 48 lb. 4 oz. of Ragwort. It died during the period of feeding, without showing definite clinical symptoms. The post-mortem lesions were typical of Ragwort poisoning, and the microscopical examination showed the presence of a parenchymatous Hepatitis. The fatty degeneration in heart and kidney were well pronounced and haemoglobinuria was also present. This represents a very acute case of *Senecio* poisoning.

Experiment No. 10.—Horse 8912, an aged grey gelding, received on the 12th October, 1914, on the station. It was utilized on the 22nd November, 1914, to test the virulency of a "T" virus to which it reacted and was thus immunized. From the 14th December, 1915, until the 25th January, 1916, the horse received daily one ounce of Ragwort mixed with food. From the 6th January, 1916, to the 25th January, 1916, the daily dose was increased to 2 oz., and from the 26th to the 17th February to 4 oz., from the 18th to the 27th to 8 oz. From the 28th February, 1916, to the 3rd March, 1916, 12 oz. daily were supplied. A total amount of 19 lb. 11 oz. were consumed in eighty days. During this period the horse showed a more or less normal temperature record with a slight disturbance in the morning from time to time, but never with any high exacerbations. The temperature kept as a rule between 100°F . and 101°F ., very rarely exceeding 101°F . From the 25th March, 1916, a slight reaction occurred which somewhat resembled Horse-sickness inasmuch as it was remittent with but slight evening exacerbations which on the 28th reached 102.8°F . On the following day the horse was examined, when it was found that the mucous membranes of the eyes were slightly orange tinged and the horse was not feeding well. On the 1st April, 1916, filling of the fossa temporalis was noted and interpreted at the time as a slight Dikkop. The horse showed a pulse frequency of 72 per minute. Examined in the morning (2nd April, 1916), the horse voided red urine. The symptoms simulating Dikkop were still present. The horse went down during the day. It died during the evening. Liver and kidney were removed soon after death. Urine was collected during the day; it was thickish, turbid, and red.

Post-mortem.—Condition was poor. Rigor mortis was present. Integument: decubitus on hips. Visible mucous membranes were yellowish and haemorrhages present in the conjunctiva. Flesh was pale and somewhat opaque. Subcutaneous tissue was slightly yellowish. A gelatinous subcutaneous infiltration was present on neck. Fossa temporalis was bulged out. Pleural cavities

contained red-stained liquid. Peritoneal cavity was normal. Diaphragm was convexly forwards, fibrous filaments were present on posterior side. Lymph glands: submandibulars were normal, retropharyngeals were hyperaemic and oedematous. The thyroids were normal. The tongue was slightly yellowish on section. The pharynx and oesophagus were normal. Respiratory organs: larynx and trachea contained foam, the mucosa was slightly yellow. The right lung was not collapsed, on section it was hyperaemic and oedematous, foam was present in bronchi. There was emphysema in anterior lobe of left lung, and slight oedema was present. The pericardium contained 500 c.c. red-stained fluid. Circulatory organs: both ventricles were empty. Epicardium showed numerous ecchymoses and extravasations. Right endocardium showed a few ecchymoses, the left one more diffuse extravasations, the valves were normal. Myocardium was pale and somewhat soft. Thoracic and abdominal aorta were normal. Intima of pulmonary artery was slightly yellowish. Liver: fibrous filaments were present on capsule, on section the parenchyma showed a green yellowish coloration, the lobules were fairly distinct, the consistence was somewhat tough. Spleen: 40×18 cms., pulp was dark red and soft; the trabeculae were indistinct. Suprarenal glands: slightly hyperaemic. Kidneys: capsule easily detached, organ on section hyperaemic, consistence normal. Stomach: mucosa of fundus slightly yellowish, a spiroptera tumour was present. Small intestines showed patches of diffuse hyperaemia. Large intestines: the mucosa of the caecum was hyperaemic and haemorrhagic infiltrations of the submucosa were present. Colon showed patchy hyperaemia. Rectum was normal. The mesentery contained no fat. The bladder was empty; its mucosa was slightly hyperaemic. The brain was slightly yellowish. The pia mater was slightly oedematous. The bone marrow was fatty and generally haemorrhagic.

Pathological anatomical diagnosis.—Traumatism of the skin. Oedema of the subcutaneous tissue and of lymph glands of head and neck. Icterus. Anaemia. Hydropericardium. Hydrothorax; oedema pulmonum and emphysema. Cloudy degeneration of heart muscle, ecchymoses of epicardium and endocardiums. Pigmentation and fatty degeneration of liver; slight tumor splenis; hyperaemia of kidneys and suprarenal glands; hyperaemia of small intestines; hyperaemia and oedema of caecum. Spiroptera tumour.

Microscopical examination of the liver.—The lobules were distinct. A striking feature was the presence of much pigment, both in the periphery and in the centre of the lobule, whilst the intermediate zone was free from it. The pigment of the periphery was bile; it was within the cells and filled the bile-ducts between the liver cells; that around the central vein was different, it was between the liver cells and within the endothelial or connective tissue cells. There was, however, also bile present in some central liver cells. The walls of the central veins were slightly thickened and hyaline, the endothelial cells of the lumen were distinctly standing out. The interlobular connective tissue was increased, both in cells and fibrillar substance. It entered between the liver cell rows of the periphery, separated the cells and atrophied them. The majority of the cells in the septa were polyblasts, the remainder were fibroblasts; there were a good many plasma cells present. Scharlach revealed some fat in the cells of the lobules irregularly distributed. Kidney: all the vessels were gorged with blood. Tubuli contorti and tubuli recti contained homogeneous deep red stained casts, sometimes epithelial cells were sticking to their surface. A homogeneous substance was also seen on some places between the tubules. The glomeruli were not affected. Scharlach showed some fine fat granules in the epithelial cells of the tubuli contorti.

Diagnosis.—Liver: pigment; icterus; emigration of round cells; atrophy of liver cells; fatty degeneration; hyaline degeneration of central veins. Kidneys: hyperaemia; cloudy degeneration; haemoglobin casts; fatty degeneration of the tubuli contorti.

Result.—This horse consumed in eighty days, until date of death, a total amount of 17 lb. of Senecio. The post-mortem lesions showed some peculiarities, typical of Horse-sickness, viz., the oedematous condition of neck and the hydrothorax and hydropericardium. It is possible that this was Horse-sickness, notwithstanding the fact that the horse was a "T" immune horse and notwithstanding that the blood taken during the disease was not virulent for a susceptible horse. The lesions during life were those of a Dikkop and those of Ragwort poisoning (haemoglobinuria), and also on post-mortem the lesions of an acute Hepatitis were present. No regeneration of bile-ducts was noted, a fact that finds perhaps an explanation by the presence of Horse-sickness that accelerated the disease.

Experiment No. 11.—Mule 9425 on the station since the 5th August, 1915, was utilized for a virus test against Horse-sickness without result. It was submitted on the 14th December, 1915, to a feeding experiment with Ragwort, receiving daily 1 oz. from the 14th to the 5th January, 2 oz. from that to the 25th January, and 4 oz. from the 26th January to the 17th February, 8 oz. from the 18th to the 27th March, and 12 oz. from the 28th March to the 6th April. A total amount of 43 lb. 3 oz. was consumed in one hundred and fourteen days. On the 8th April, 1916, the experiment was discontinued and the animal was killed. During the experiment the temperature kept normal, with an occasional exacerbation of no importance. No symptoms were noticed during this period, the animal kept healthy.

Post-mortem.—The autopsy was made two hours after death, the condition was good. Rigor mortis was not complete. The integument was intact. Blood escaped from nostrils (bullet). The flesh showed nothing unusual, the subcutaneous tissue contained some fat. The pleural cavities were normal. The diaphragm convexly forwards, fibrous filaments were present on peritoneal side. Peritoneal cavity showed nothing unusual. Submandibular and retropharyngeal lymph glands showed nothing unusual. Thyroids were small. Bronchial and mediastinal lymph glands contained black pigment. Tongue, oesophagus, and pharynx showed nothing unusual. Cervical trachea and larynx showed nothing unusual. Lungs were collapsed, the pleura was smooth, in both anterior lobes and along the margin some emphysema was present, under the pleura of the right lobe were some bluish irregular patches; the lung tissue was elastic; bronchi and thoracic trachea showed nothing unusual. Pericardium contained 10 c.c. clear straw-coloured fluid. Heart was in systole. The ventricles were empty. The epicardium was normal. Endocardiums were smooth and glistening, a few small haemorrhages were on left endocardium. Myocardium was transparent. Foramen ovale was closed. Intima of coronary arteries was normal. Pulmonary vessels showed nothing unusual. Intima of aorta was smooth. Intima of anterior mesenteric artery was thickened and in parts roughened. Liver was somewhat small, the margins were sharp, right lobe on peritoneal side showed some fibrous patches (4.6½ cms.) and filaments on the capsule. On section parenchyma was slightly dark in colour, lobules fairly distinct, central veins filled with blood, some lobules appeared whitish; consistence was normal. Spleen: 41 × 23 cms., pulp was dark brown, dry, trabeculae were distinct, follicles were not visible. Left and right suprarenal glands showed fat in the cortex. Kidneys were rich in fat, capsule was easily detached, left kidney on section was slightly dark, consistence showed nothing unusual, right kidney was darker, especially the intermediate zone. Stomach contained food, mucosa showed nothing unusual. Intestines were normal. The mesentery was rich in fat; the lymph glands showed no abnormalities. Some turbid urine was in the bladder. Ovaries were small. Vagina and uterus showed nothing unusual. The brain showed a haemorrhage under the dura mater (bullet); the ventricles were normal. The cerebellum showed nothing unusual. Bone marrow of humerus and femur was fatty, in parts it was red.

Pathological anatomical diagnosis.—Anthraxis of lymph glands. Ecchymoses of left endocardium. Emphysema pulmonum and atelectasis. Atrophy of liver. Endoarteritis chronica et productiva of the mesentery.

Result.—This mule was fed over a period of one hundred and fourteen days on Ragwort, consuming a total amount of 43 lb. It did not show any symptoms of Ragwort poisoning. It was killed one day after the last feed and on post-mortem did not show any signs of parenchymatous Hepatitis. The microscopical examination was unfortunately missed.

III. EXPERIMENTS IN KOKSTAD WITH GREEN PLANTS.

Experiment No. 12.—These experiments were carried out by the Government Veterinary Surgeon of that district and the notes were made from his reports.

Horse 10394, an aged dark brown gelding, obtained from the Defence Force on the 19th June, 1916. It was immunized against Horse-sickness. On the 21st September, 1916, it was sent to Kokstad for a Ragwort feeding experiment with freshly collected green plants (*Senecio latifolius*). From the 28th September, 1916, to the 12th October, 1916, it shared a common manger with horse 9614, and together they consumed 15½ lb. in twelve days. From the 16th to the 18th day it ate ½ lb. daily; on the 19th, 1 lb.; on the 20th, ½ lb.; on the 21st and 22nd, ¾ lb. daily; 23rd, ½ lb.; 24th and 25th, 1 lb. daily; 26th, ½ lb.; 27th, 1 lb.;

28th, $\frac{3}{4}$ lb.; 29th, 1 lb.; 30th to the 32nd, $\frac{1}{2}$ lb. daily; 33rd, 1 lb.; 34th, $\frac{1}{2}$ lb.; 35th, 1 lb.; 36th, $\frac{1}{2}$ lb.; 37th, 1 lb.; 38th, $\frac{1}{2}$ lb.; 39th, 1 lb.; 40th and 41st, $\frac{1}{2}$ lb.; 42nd, 1 lb.; 43rd and 44th, $\frac{1}{2}$ lb.; 45th, 1 lb.; 46th, $\frac{1}{2}$ lb.; 47th, 1 lb.; from the 48th to the 50th, $\frac{1}{2}$ lb. daily. For the next three days it did not eat any plant and was destroyed on the 55th day. (22nd November, 1916.) The horse consumed a total amount of 25 $\frac{1}{2}$ lb. in thirty-eight days, plus the 15 $\frac{1}{2}$ lb. shared with horse 9614 in twelve days (32 lb. 14 oz. in fifty days).

Symptoms.—18th November, 1916.—Conjunctiva was jaundiced. The rectal temperature 98.6° F. The horse had refused food since last evening, with the exception of a little lucerne. 20th November, 1916, 11 a.m.—The horse was showing abdominal pain, pawing with the fore feet, it was inclined to crouch, it kicked at the belly and lay down, its head was constantly hanging down towards the left, sometimes turned towards the abdomen. A normal amount of faeces was passed, drier than usual, yellow in colour, inoffensive in odour. On listening at the sides one could hear constant rumbling of the bowels. There was a muscular trembling in hindquarters and forearm. The conjunctiva remained jaundiced and the veins running over the membrana nictitans were distended; these vessels had the appearance of whitish blue cords. When the horse was led out into the yard it was inclined to nibble at the grass, but was very weak. The temperature on the 20th had been at 8 a.m. 102.2° F., at 1 p.m. 102.4° F. A further symptom noted was that of being inclined to rest the head on the manger or against it. In the evening the horse passed discoloured urine. The following morning numerous ecchymoses were present on the conjunctiva, the corded blood-vessels had disappeared. At midday the temperature was 104.2° F. The horse refused to feed in the stable and would not even take freshly cut lucerne, but as soon as it was turned out it grazed fairly well. 250 c.c. of a 2 per cent. trypan blue solution were injected. At 8 a.m. on the 22nd November, 1916, the temperature was 101.6° F. The horse fed well in the veld. At 1 p.m. its temperature was 101° F. The horse seemed dazed and held the forage in its mouth without attempting to eat or swallow it. It voided a brownish urine in jets and seemed at times semi-conscious. At 7 p.m. the horse was destroyed.

Post-mortem.—Post-mortem revealed the presence of a large collection, about four pints, of partly coagulated blood connected with both kidneys, each kidney had an extraordinary appearance as though a portion of the surface had been worn away. The bladder contained about two pints of brown urine. The liver was distinctly cirrhotic. The large colon showed a lesion, about 1 $\frac{1}{2}$ inches in diameter, which was thickened and oedematous. The ingesta throughout had an offensive odour. The heart appeared enlarged, the cavities were normal in capacity, and no ecchymoses were present on the external surface. (Unfortunately the post-mortem had to be made by lamp light and was somewhat crude.)

Naked-eye examination of the liver.—A piece fixed in formalin had a distinctly greenish appearance and showed darker green areas in a lighter green background, the cut surface thus appeared somewhat mottled. The outlines of some reticular meshwork could be made out without recognizing any further details.

Pathological anatomical diagnosis.—Subcapsular haemorrhages in kidneys. Icterus. Haemoglobinuria. Cirrhosis. Ecchymoses in epicardium.

Microscopical examination.—The lobulation could be made out from the position of the central vein and the septum. The lobules, however, had an abnormal appearance and various stages of desintegration could be recognized. In the most advanced ones practically no liver cells were left. Their place was occupied either by a homogeneous substance, remainders of liver cells, and between them scattered or in clusters round cells and red corpuscles. There was much pigment about of a brownish colour, either free or in remnants of liver cells. The periphery of some lobules showed a zone of liver cells having various shapes; they were vacuolated and contained pigment. Some lobules still showed the radiating picture of cell rows, some of the rows or pieces of the rows were missing, thus causing a patchy appearance. None of the liver cells left were intact, they were faded or vacuolated or contained pigment. Sometimes small foci of fairly closely packed round cells and red corpuscles were seen. Much pigment was seen in the cells bordering the septa. There was no increase of connective tissue in the septa, an increase of small round cells (lymphoid type), however, was present. In the periphery of some of the lobules large multinuclear cells could be seen, the protoplasm being lighter in colour. They were rare

and appeared as budding bile-ducts. Kidney: extensive fatty degeneration of the tubuli contorti was noted, the fat granules being of large size. In the Scharlach-stained section red radiating stripes were seen separated by the tubuli recti. In the Haematoxyline-eosine section were vacuoles of different sizes. Between the fatty tubuli contorti were pieces of tubules the protoplasm of which was apparently intact and the nuclei deeply stained. Only in one or two places were seen an accumulation of round cells. The larger blood-vessels (arteria lobularis) contained red corpuscles very closely packed. They were also found in the lumen of some of the tubules and the interlobular capillaries were also filled with them. There were homogeneous exudate and blood in Bowman's capsule. The lumen of the tubuli contorti was filled with homogeneous, somewhat cloudy substance. Under the capsule was a thick layer of blood, the corpuscles being closely packed.

Diagnosis.—Liver: fatty degeneration, atrophy and necrosis of the liver cells, slight cirrhosis, regeneration of bile-ducts, emigration of round cells, pigmentation. Kidneys: oedema, fatty degeneration and necrobiosis of epithelium of tubuli contorti, subcapsular haemorrhage.

Result.—This horse consumed during a period of fifty days 32 lb. The horse was killed whilst it showed the typical symptoms of Ragwort poisoning.

Experiment No. 13.—Horse 9614, a six-year-old brown gelding, obtained from the Defence Force in August, 1915. From the 24th January, 1916, to the 24th April, 1916, it was dipped in an arsenic bath every seventh day. Later it passed the immunization against Horse-sickness, showing the symptoms of a Dikkop. On the 21st September, 1916, it was sent to Kokstad for a Ragwort feeding experiment. For the first fifteen days (28th September, 1916, to the 12th October, 1916) this horse shared a common manger with horse 10394, and together they consumed 15½ lb. The horse ate 1 lb. on the 16th day; on the 17th and 18th, ¾ lb. daily; on the 19th, 1 lb.; on the 20th and 21st, ¾ lb. daily; on the 22nd and 23rd, ½ lb. daily; from the 24th to the 27th, 1 lb. daily; on the 28th, ¾ lb.; on the 29th, 1 lb.; and the 30th, ½ lb.; on the 31st and 32nd, ½ lb. daily; on the 33rd and 34th, 1 lb. daily; on the 35th, 1 lb.; 36th, ¾ lb.; 37th, 1 lb.; 38th, ½ lb.; from the 39th to the 53rd, 1 lb. daily; 54th, ½ lb.; 55th, 1 lb.; 56th, ½ lb.; and on the 57th and 58th, 1 lb. daily; making a total of 37 lb. from the 16th to the 58th day. The horse was returned to Onderstepoort on the 27th December, 1916, in good health. The horse was still alive at the end of November, 1917.

Result.—This horse was the mate of horse 10394 with which it shared for some time the manger. It consumed more Ragwort during this experiment than its mate, viz., about 45 lb. in fifty-eight days. It did not develop any symptoms during the period in which it was under observation.

IV. FEEDING EXPERIMENTS WITH DRIED *Senecio latifolius* IN ONDERSTEEPOORT.

Experiment No. 14.—Horse 9200, an aged brown gelding, purchased on the 2nd March, 1915, was immunized against Horse-sickness, passing through a Horse-sickness reaction. On the 16th March, 1916, it was submitted to a Ragwort feeding experiment, and received from the 16th March to the 6th April 12 oz. daily. During this period the temperature was irregular, having on one occasion a fever exacerbation to 104° F. in the evening of the 28th March. The feeding was discontinued for a time. It was again continued from the 28th April to the 18th of May, the horse receiving 8 oz. daily. The temperature after the second feeding was very irregular at the beginning, showing a fever exacerbation to 106° F. on the 30th April. From the 3rd May the temperature returned to and remained normal. The feeding was discontinued on the 18th May, 1916, the horse having consumed a total amount of 27 lb. in forty-three days. It was utilized in an arsenic experiment on the 16th July, 1916, in which it died.

Result.—27 lb. of *Senecio* fed over a period of forty-three days did not produce any disease within fifty-nine days after discontinuation of the feeding. At the time of this experiment it was not realized that animals could go down with Ragwort poisoning, even after a long period.

Experiment No. 15.—Horse 3751, a chestnut mare, obtained from the Standerton Stud Farm, arriving at Onderstepoort on the 16th April, 1908. The horse was immunized and passed through the Horse-sickness reaction. It was fed on Ragwort mixed with its ordinary food, receiving 12 oz. daily from the 20th March, 1916, to the 6th April, 1916, and from the 28th April, 1916, until

the 18th May, 1916, 8 oz. On two occasions there was a disturbance in the temperature. An exacerbation was noted on the 1st May to 104° F. and again on the 7th and 8th to 102.8° F., otherwise the temperature remained normal. The feeding of Ragwort was discontinued after the 18th May, 1916. The horse consumed a total amount of 24 lb. in thirty-nine days. The horse died on the 21st September, 1916, in an arsenic experiment.

Result.—24 lb. of *Senecio* spread over thirty-nine days did not produce any disease in this horse. The animal had lived up to one hundred and fifty-six days after discontinuation of the feeding, a period sufficiently long, according to later experience, during which all deaths subsequent to feeding had occurred.

Experiment No. 16.—Horse 7298, an aged brown gelding, obtained from the Department of Agriculture, Orange Free State, and arrived at Onderstepoort in December, 1916. On the 23rd January, 1917, it was placed into a Ragwort feeding experiment, receiving 2 oz. daily mixed with ordinary food until the 28th. From the 29th January to the 7th February it received 3 oz., from the 8th to the 19th 4 oz., from the 20th February to the 3rd March, 6 oz. daily, when the feeding was discontinued for a few days. From the 6th to the 27th it again received 6 oz., from the 28th March to the 3rd April 1 lb., from the 4th to the 28th 6 oz., from the 29th to the 30th 8 oz., and from the 1st to 7th May 8 oz. daily, after which the feeding was discontinued; the horse having consumed a total amount of 39 lb. 4 oz. in one hundred and three days. On the 16th May this horse was immunized against Horse-sickness, receiving as a first injection virus Tzaneen of the 15th generation, and seven days later virus of Onderstepoort, Tzaneen, and Bulawayo, succeeded by serum two days later. A very slight reaction was noted on the tenth day, the evening temperature being 101.2° F., and on the eleventh 102° F., this was the highest temperature observed. On the 26th May, 1917, the horse was noted to be ill, its head was slightly hanging, the eyes were staring, the mucous membranes of the eye appeared orange tinged and ecchymosed. There were abrasions on the skin of the supra-orbital process and on the zygomatic arch and of the frontal region. The heart impulse was fairly pronounced and a metallic timbre was noticeable at the end of the first sound. When the horse was left alone it had a tendency to push forwards; it was not feeding well; an occasional tremor was noticed in the triceps brachii. After the horse was placed in a loose box it was noticed to go down and remained in the sternal position the whole day long. During this time it was seen to keep some food in its mouth, not chewing it. On the 27th the horse was looking somewhat brighter, the pulse was 64, the impulse still well noticeable. When walking the horse occasionally swayed and the walk was somewhat clumsy, unusually long steps being taken. On the 28th May, 1917, the pulse was 68, the impulse was very marked, the sounds well pronounced, the first with a distinct metallic timbre. The head was kept hanging, the fore-quarter was slightly pushing forwards, the horse dragged the hind legs and swayed slightly when walking. The urine collected on this day showed bile pigments in considerable amount. In the morning of the 29th the horse was not feeding, it was observed to rest its head on the manger, the pulse was 84 and small, the eyes were ecchymosed. The area of impulse was extended backwards; a peculiar vibration of the chest wall above the heart could be felt at each contraction. The horse was walking with the head down, it swayed in turning, it dragged the legs. 30th May, 1917.—In the morning the horse was found stretched out, lying quiet, showing a dyspnoea. It died during the day.

Post-mortem.—The autopsy was made two hours after death. The condition was moderate. The abdomen was slightly sunken. Rigor mortis was just beginning. Abrasions were present above both eyes. Tongue was hanging out. Visible mucous membranes of mouth were pale yellowish. The lips were slightly swollen. There was a moist discharge from the nostrils. The eyes were half closed. The anus was closed. The blood was liquid, it stained very badly and had a brownish tinge. The subcutaneous tissue had a yellowish tinge. The muscles were dry and had a brownish tinge. Parotids and submaxillary glands were somewhat moist. Lymph glands: retropharyngeals were slightly enlarged and on section haemorrhagic, the surrounding connective tissue was moist, upper cervicals showed some gelatinous infiltration. The thyroids were normal in size and on section dark in colour. At the base of the tongue were two young *Gastrophilus* larvae. The tonsillae showed wide pits. The guttural pouches showed injected vessels and ecchymoses. The mucosa of the pharynx was congested. Tongue and oesophagus were of usual appearance. Mucosa of trachea was smooth and glistening. The tissue around the pharynx was

unusually moist. Serosa of peritoneal cavity was smooth and glistening, somewhat brownish tinged. Diaphragm was convexly forwards. Numerous fibrous filaments were present on posterior side. Costal pleura was smooth and glistening. Mediastinal glands were haemorrhagic, the bronchial glands showed nothing unusual. Lungs were collapsed; left lung had fibrous filaments and emphysema along the ventral border. Blue discoloration was present under the pleura and numerous ecchymoses. Lung was elastic and tissue on section moist. Intima of the vein was smooth. In right lung were numerous ecchymoses and on pleura a few fibrous filaments. Well pronounced emphysema was present in apex. Tissue was elastic and moist. Intima of vein was smooth and glistening. The bronchus contained a little froth. Intima of artery was smooth. Thoracic trachea was of normal appearance. In the upper portion of the oesophagus were three gastrophilus larvae. Lower cervical and anterior mediastinal lymph glands were diffusely haemorrhagic, and the supporting connective tissue was oedematous. Intima of aorta was smooth and glistening. In the walls of the pericardium was ochre yellow fat, the vessels injected. It contained 150 c.c. of a brown clear liquid, which on shaking showed a greenish tinge. The parietal serosa was smooth and glistening. Circulatory organs: both ventricles and atria contained uncoagulated blood, right ventricle was in diastole, the ostium was open for four fingers, a fair amount of fat was at base of the heart and in sulci longitudinales; epicardium was smooth, some ecchymoses were found along the longitudinal and transverse sulci; the colour was light brown, sugillations were seen in septum and yellowish superficial infiltrations, sugillations were also in right auricle; left ventricle was also in diastole; the ostium was open for four fingers; muscoli papillares showed sugillations and petechiae, also the septum; bicuspid valve showed ecchymoses and had a yellowish colour; semilunar valves were normal. Coronary arteries: intima was smooth and glistening. Myocardium, left 3.5 cms., right 2 cms. of grey appearance, somewhat opaque; septum looked as if boiled, consistence was somewhat soft. Periportal glands were much enlarged; on section some liquid escaped. The glands were brown in colour, their consistence was soft. Size of liver was normal. On anterior surface of right lobe were found numerous fibrous filaments and a white fibrous patch, 16×4 cms. On left lobe were only a few fibrous filaments. The capsule of the visceral surface was smooth. The lymph vessels were prominent. The colour was mottled brown. The section had a brown yellow appearance, it was distinctly nutmeg. The centres of the lobules were red, the periphery had a grey appearance. In parts the septa were visible. Bile was seen in the ducts. Consistence of the liver was firm. In the right lobe a fair amount of blood was present. The pancreas was rich in blood, moist, and of normal consistence. The splenic lymph glands were somewhat enlarged and moist. Some liquid escaped on section. Spleen: 47×24 cms. Some fibrous filaments were present on the capsule, otherwise it was smooth. On section the pulp was dark brown. The trabeculae were visible, the follicles were not visible. Consistence somewhat soft. Suprarenal glands appeared unusually large, the cortex was yellow, the medulla haemorrhagic. Left kidney: Fat was present in the capsule, which was easily detached, the organ was fairly rich in blood, the intermediary zone was streaked red and grey, the cortex was opaque and striated, consistence was slightly soft. The capsule of the right kidney showed a haemorrhagic infiltration and was easily detached; the parenchyma was somewhat opaque, it was rich in blood, the cortex was striated red and yellow, the intermediary zone showed red stripes, the consistence was slightly softened. Fundus of stomach was diffusely hyperaemic, the mucosa was thickened, black coagulated blood was present on the surface. Cardiac mucosa contained a few gastrophilus larvae. Pylorus: black streaks and patches in the mucosa. Duodenum was brownish discoloured. Small intestines: large haemorrhagic patches of one to two feet in length were present throughout the small intestines. Caecum and colon showed diffuse haemorrhagic infiltration in a thickened mucous membrane. Small colon showed diffuse hyperaemia and a mucous deposit. Rectum was filled with faeces, the mucosa was patchy red. Mesenteric lymph glands slightly moist and pinkish. Abdominal aorta: intima smooth and glistening. An aneurism was present in the arteria ileo-caeco-colica. The bladder contained turbid yellow urine, which had a greenish tinge. The vessels of the mucosa were injected. Examination of urine: marked bile reaction on 28th and 29th May. On day of death (30th May, 1917) the bile reaction was still marked and metahemoglobin reaction faint. Urine slightly decomposed and NH_3 therefore not determined. Brain was slightly brownish in colour and moist, the vessels were injected. Teeth were normal. Bone marrow in femur was distinctly red. The marrow of the humerus was fatty with a haemorrhagic focus. Body weight,

315.5 kg.; right lung, 3.3 kg.; left lung, 3.6 kg.; heart, 3.16 kg.; liver, 6 kg.; spleen, 1.5 kg.; left kidney, .8 kg.; and right kidney, .8 kg. Liver weight: body weight = 1 : 52.6.

Pathological anatomical diagnosis.—Traumatism on head. Icterus. Anaemia. Dissolution sanguinis. Hyperaemia of pharynx and larynx. Perilaryngeal and pharyngeal oedema. Hyperaemia of guttural pouches. Oedema of the salivary glands. Oedematous and haemorrhagic lymph glands of pharynx and mediastinum. Pleuritis fibrosa and emphysema pulmonum. Slight oedema pulmonum. Hydropericardium. Cloudy and fatty degeneration of heart. Ecchymoses in both ventricles. Perihepatitis fibrosa, stasis in lymphatic vessels of Glisson's capsule. Stasis and fatty degeneration of the liver. Stasis and fatty degeneration of the kidneys. Fatty degeneration and stasis of adrenal glands. Slight tumor splenis. Perisplenitis fibrosa. Gastritis and enteritis haemorrhagica. Gastrophilus larvae on radix of the tongue, in oesophagus and stomach. Aneurism of arteria ileo-caeco-colica. Methaemoglobinuria.

Microscopical examination.—Liver: the section had a mottled appearance due to the presence of round red spots in a whitish ground substance, some of the spots were confluent, the white substance was forming a kind of meshwork. Under a low power the septa could be seen. The central portion of the lobules was occupied by blood which also distended the adjoining capillaries, so that fairly large pools were formed. Here the liver cells were absent or less numerous. Some of the central veins showed thickened walls, but only to a slight degree. The liver cells were pale and vacuolated, vacuoles of different sizes being present. In the Scharlach-stained section the presence of much fat was shown. Between the fat globules and the blood corpuscles the liver cells almost disappeared. Also the bile-ducts in the septa showed small fat droplets in their epithelial lining. No budding bile-ducts could be made out, although at one place in a thick septum containing large veins and arteries, around a bile-duct a number of small ducts cut crossways were seen to be radiating off. The septa were slightly thickened, due to the presence of fibroblasts and fibrillar bundles and some homogeneous substance between them. There was also a good amount of yellow pigment present. An increase of white corpuscles was present—they were scattered about. Kidneys: some of the glomeruli had thickened capsules and some were atrophied. The cells of the tubuli contorti of the peripheral portion of the cortex showed much vacuolation. Scharlach-stained sections showed the presence of fat globules in the vacuolated portion. There was a fair amount of blood present. In the adventitia of some vessels were small clusters of round cells. The thinner tubules of Henle's loops also showed fat infiltration in the epithelial cells. Heart: right wall, already the naked-eye vision showed in the Scharlach-stained section a diffuse staining of fibres. In some parts the fibres were broken and between the broken substance was seen an increase of leucocytes. The septum: the diffuse red discoloration was even more intensive. There were several patches where the fibres were broken and leucocytes were present, mingled with the fragments. There were also patches of fibroblastic tissue replacing the fibres. In many places the clefts between the fibres were occupied by leucocytes. Left wall: similar conditions were noted as in the septum, but the fatty degeneration was not so intensive. Parotis: there were patches of alveoli showing the fat globules in their epithelial lining and these patches were most frequently situated near a duct, the epithelial lining of the duct itself showed fat globules. Submaxillary glands: also here the epithelial lining of a number of alveoli took the Scharlach stain. The mixed alveoli seemed to be attacked by preference, so that the light transparent portion of the alveolus was surrounded by a red ring of fatty cells. The ducts showed no fat. Suprarenal glands: Scharlach-stained sections of the cortex showed macroscopically a diffuse reddening. Muscles: these showed a good number of fibres undergoing fatty degeneration. They seemed to be evenly distributed, so that the section had a somewhat patchy appearance in the pieces cut crossways. In the sections cut longitudinally the degenerated fibres were separated from each other by intact ones. Rarely were numbers of degenerated fibres seen running together.

Diagnosis.—Stasis, pigmentation, and fatty degeneration of the liver, slight cirrhosis. Emigration of leucocytes. Fatty degeneration of the kidney. Nephritis chronica interstitialis. Fatty degeneration of parotis, submaxillary glands and suprarenal glands. Myocarditis fibroblastica and myodegeneratio adiposa. Fatty degeneration of the muscles.

Result.—This horse was not immunized against Horse-sickness before the feeding experiment with Ragwort was started, but immunized nine days after discontinuation of the feeding. The horse went through a mild Horse-sickness reaction. During the reaction the symptoms of Ragwort poisoning appeared and the animal died, viz., twenty-three days after discontinuation of the feeding. The lesions of a Hepatitis were present, but more those of an acute fatty degeneration, which were also present in the kidneys and other organs. It is thus possible that the Horse-sickness reaction, though in itself quite mild, contributed to the death of the horse, which was then suffering from the effects of Ragwort poison.

Experiment No. 17.—Horse 7299, an aged brown gelding, obtained from the Orange Free State Department of Agriculture, arriving at Onderstepoort in December, 1916. On the 23rd January, 1917, it was placed into a Ragwort feeding experiment, receiving daily, mixed with its food, 2 oz. of Ragwort until the 29th. From the 30th January to the 7th February it received daily 3 oz., from the 8th to the 19th 4 oz., from the 20th February to the 3rd March 6 oz. The feeding then was stopped for a few days. It again received 6 oz. daily from the 6th to the 27th, from the 28th March to the 3rd April 1 lb., from the 4th to the 28th 6 oz., and from the 29th April to the 7th May 8 oz. daily. The feeding was then discontinued, the horse having consumed in all 39 lb. 3 oz. in one hundred and three days. This horse was immunized against Horse-sickness on the 16th May, 1917, receiving for the first injection Tzaneen virus of the 15th generation, for the second, seven days later, virus "O" and serum on two successive days, the 7th and 9th. The horse developed a very slight reaction, which was accompanied with the symptoms of Dikkop, from the 16th to the 20th day a fever reaction was present, during this period the conjunctiva had a brownish tinge, and the pulse varied between 60 and 80. The impulse was well pronounced. The diagnosis of pernicious Anaemia was made, but the animal recovered from this attack. (The virus utilized for the second injection turned out to be a pernicious Anaemia virus.) Subsequently until the 10th August no further symptoms were noticed, the temperature, although at times slightly irregular showed no marked exacerbations. On the morning of the 10th August the horse was found down; it was lifted and transferred to a loose box in which it stood until the afternoon, when it went down again, lying stretched out and unable to regain a sternal position, lying quiet with half-closed eyes. It died the same evening. This horse had during the last few weeks considerably fallen off in condition.

Post-mortem.—Condition fair. Rigor mortis not present. Visible mucous membranes were yellowish. Natural openings normal. Blood not coagulated and thin. Flesh was pale, subcutaneous tissues were stained yellow throughout. Mandibular lymph glands were slightly hyperaemic, the retropharyngeals moist and markedly hyperaemic, the upper cervicals were moist. Thyroids were dark in colour. Tongue muscles were yellow in colour. The oesophagus was normal, also the pharynx. In the trachea was a small quantity of yellowish mucus. The larynx was markedly hyperaemic. Bronchial and mediastinal lymph glands were moist and showed marked hyperaemia. Pleura of left lung showed fibrous filaments and numerous petechiae on anterior lobe. The margo acutus of the corpus pulmonum showed commencing atelectasis. This portion was reddish green in colour and firm in consistence. Rest of the lung showed stasis. In the bronchi was a small quantity of yellow mucus. The artery was empty. In the pleura of the right lung were a few fibrous filaments. Tissue on section showed marked stasis. Mucosa of bronchus showed numerous petechiae, the artery contained uncoagulated blood. Thoracic trachea showed the mucous membrane deep yellow in colour. The oesophagus was normal. Intima of aorta was yellow. The pericardium contained 100 c.c. reddish yellow turbid fluid. Circulatory organs: right ventricle and atrium were empty. Ostium admitted the hand. Left ventricle was empty, ostium admitted four fingers. Epicardium showed a few petechiae on the transverse sulcus of the left ventricle. Right endocardium was normal. The valves showed a slight gelatinous infiltration. The left endocardium showed a few echymoses on musculi papillares (not recent). Left myocardium was 4 cm., the right 2 cm. thick. On section it was pale brownish yellow in colour. Liver capsule showed a few fibrous filaments, otherwise it was smooth and glistening. On section a very distinct lobulation was present in the liver tissue. Centres of lobules were greenish brown in colour, and the interlobular connective tissue was very distinct. The consistence was firm. The pancreas was normal. Spleen measured 36 × 19 cms. Capsule was normal. The colour of the pulp was dark. On section trabeculae were

distinct. The left and right suprarenal glands were normal. The left kidney capsule was easily detached. On section the glomeruli were distinct. The colour was dark yellowish brown. Right capsule was also easily detached. Organ on section showed same appearance as the left. Stomach: the fundus was normal. Numerous *Gastrophilus* larvae, both equi and pecorum were present. In cardiac portion a small patch of hyperaemia was seen. A spiroptera tumor, the size of a pigeon's egg, was present. Duodenum was normal. Small intestines were slate coloured. Large intestines: the caecum was diffusely hyperaemic. Colon was dark slate coloured, also the floating colon and rectum. Mesenteric lymph glands were markedly hyperaemic. Intima of abdominal aorta was yellow. The bladder was yellowish brown, the urine was turbid. The brain was normal. Bone marrow of humerus showed a haemorrhagic infiltration. Teeth were in good condition. Body weight, 299.6 kg.; right lung, 3.82 kg.; left lung, 3.46 kg.; heart, 2.5 kg.; liver, 5.6 kg.; spleen, 1.52 kg.; kidneys, 2.15 kg. Body weight : liver weight = 1 : 53.5.

Pathological anatomical diagnosis.—Icterus. Anaemia. Hyperaemia and oedema of lymph glands and of the thyroids. Atelectasis and stasis of lungs. Pleuritis fibrosa. Slight hydropericardium. Bronchitis catarrhalis and haemorrhagica. Ecchymoses and petechiae on epicardium and endocardium. Fatty degeneration of myocardium. Cirrhosis and pigmentation of liver. Perihepatitis fibrosa. Fatty degeneration of kidney. Hyperaemia of intestinal tract. *Gastrophilus* larvae in stomach.

Microscopical examination.—Liver, right lobe: under low power (16×) the section had a patchy appearance, due to deeper coloured centres surrounded by paler coloured peripheries. In the centre again different shadings were noted. Under higher power (65× and 125×) the central portion was seen to consist of a few darker stained liver cells or portions of liver cell rows. Between them the spaces were filled with red corpuscles and vacuolated liver cells and remnants of liver cells, in some of these was present a brown yellowish pigment. There were also round cells present, and in some places fusiform cells with spindle-shaped nuclei. Surrounding this central portion was a zone in which young bile-ducts were seen, more or less radiating towards the periphery, they were embedded in a thin transparent tissue, consisting of fusiform cells with spindle shaped nuclei and also round cells. In this zone were also found vacuolated and faded liver cells and remnants of such, some contained pigment. The bile-ducts consisted of lighter coloured cells, some were arranged in groups with fairly closely packed vesicular nuclei, some of the groups showed a distinct lumen. The periphery, viz., the septum showed comparatively few of these budding bile-ducts. It consisted mainly of thin transparent connective tissue meshes with round cells, some of which distinctly of polyblastic type and lymphocytes. There was a homogeneous substance present, that apparently was soaking through all the tissue. In some places a distinct increase of connective tissue bundles was seen and in other parts bundles of fibroblasts. Some of the bile-duct cells assumed the arrangements of liver cell rows, and also their colour had become darker, suggesting a transition of these bile-ducts into liver cells. The central vein as a rule was not thickened, although in some places it appeared that the wall was enlarged by the homogeneous substance separating the elements of it. The Scharlach stain showed large fat globules in the remaining liver cells of the centre and small droplets in the bile-ducts; usually in the periphery of the centre most of the fat was seen. The light brown coloured pigment, consisting of fine grains, showed up well in the Scharlach-stained section, and it was scattered all over the lobule, but in some parts in cells bordering the septum. Middle lobe: the conditions were almost identical, there seemed to be less pigment and in some parts less bile-ducts, whilst in the interlobular septum round cells were more conspicuous than in others. Left lobe: identical conditions as in right and middle lobes. In some places clusters of small round cells were rather conspicuous, and in parts groups of young bile-ducts were seen penetrating into the periphery of the broken-up lobule. Kidney: right. In the cells of the tubuli contorti fairly large vacuoles were seen. In the tubules and between them as well as in the glomerular capsules was seen a homogeneous substance. Many of the nuclei of the epithelial lining were compact and dark stained (pycnotic). The tubuli recti showed less vacuoles and the nuclei were pale. In the Scharlach-stained section the fat globules were large in the tubuli contorti, rare and small in the tubuli recti. Left kidney: similar conditions were present. In some tubuli contorti no nuclei were seen. Heart, right wall: practically all the fibres were tinged red and in the greater portion with the same intensity, so that the section by naked-eye inspection showed up red.

In most fibres the globules were very fine and dust-like and obscured the striation, in some places, however, they were confluent and formed globules. In some places the fibres appeared homogeneous as in a process of dissolution, the fat globules were outside the fibres and the fibres missing in patches. The patch was filled with transparent substance. In parts there was a slight increase of leucocytes. Septum cordis: the fatty degeneration was present to a much lesser degree; there were, however, patches in which the white corpuscles seemed to be increased. Left wall: the fatty degeneration was more in a streaky distribution and not so intensive as in the other two parts of the heart. Spleen: the sinuses were filled with red corpuscles; there was some bright yellow pigment present in one of the trabeculae. Suprarenal glands: the cortex stained with Scharlach was diffusely red, and on microscopical examination the cells were seen to be occupied by large fat globules, only the nucleus sticking out of an individual cell. In the Haemotoxyline-eosine stained section the cortex was pale pink and freely vacuolated. The capillaries between the cell rows were filled with red corpuscles. Muscle: individual fibres showed up diffusely red stained with Scharlach. The cross section of a bundle therefore had a patchy appearance. There were a few sarcosporidia*present.

Diagnosis.—Atrophy and fatty degeneration of liver cells. Regeneration of bile-ducts, formation of granulation tissue, emigration of round cells. Fatty degeneration of kidney. Myocarditis and fatty degeneration of heart and muscle. Fatty degeneration of suprarenal glands. Fatty degeneration of the muscles of the skeleton. Pigmentation of spleen.

Result.—This horse consumed in one hundred and three days a total amount of 39 lb. of Ragwort. It was not immune to Horse-sickness. It was immunized nine days after the discontinuation of feeding. It developed an attack of pernicious Anaemia from which it recovered. The horse died ninety-six days after discontinuation of feeding, having shown previously a falling off in condition. The course of the actual disease was very rapid, the animal being found down one morning without having given any other warning. The lesions of parenchymatous hepatitis were well pronounced and regeneration of bile-ducts was present to some extent. Also lesions of Senecio poisoning in other organs were well developed. The interesting feature in this horse is the fact that it died of Senecio poisoning ninety-six days after discontinuation of feeding, and that after such a long interval the lesions of an acute poisoning were present. In its rapid course it clinically somewhat resembled acute liver atrophy.

Experiment No. 18.—Horse 10704, an aged bay gelding, purchased and arrived at Onderstepoort on the 1st January, 1916. It was utilized for a virus reservoir experiment for Horse-sickness. On the 23rd January, 1916, it was used in a Ragwort feeding experiment, receiving daily until the 29th 2 oz. of Ragwort mixed with food. From the 30th January, 1916, to the 7th February 3 oz. daily, from the 8th to the 19th 4 oz. daily, from the 20th February to the 3rd March 6 oz. daily, when the feeding was stopped for a few days. From the 6th to the 27th it again received 6 oz. daily, from the 28th March to the 3rd April 1 lb. daily, from the 4th to the 28th 6 oz. daily, and from the 29th April to the 26th May 8 oz. daily, when the experiment was discontinued, the horse having eaten 48 lb. 11 oz. in one hundred and twenty-two days. This horse was not immunized against Horse-sickness. On the 7th June, 1917, a fever reaction was noted, rising to 103.6° F. on the 8th, which was the maximum, and returning to 99° F. on the 12th. For the next two days the temperature was below 100° F. and rose to 103° F. in the morning of the 15th, on which day it died. Since the 8th June, 1917, the horse had been noted to be visibly ill, when standing, its head was hanging. The walk was sluggish, it showed stiffness in the front legs, the hind legs were slightly swollen. The cardiac impulse was very distinct and a metallic timbre was noticed. On the 9th the pulse numbered 96, the impulse pronounced and somewhat vibrating; the respirations numbered 18, they were of a slightly abdominal type. The horse was very dull, tucked up and a loss of condition was evident. The urine collected on this day had a dark brownish colour but was clear, no albumen was present. On the 10th the pulses numbered 104, the impulse was visible, coinciding with the first sound of the heart a somewhat chafing noise was noted, and with the second a metallic timbre, between the two sounds occasionally a crackling noise could be heard. The respirations numbered 22, the conjunctiva was brown. On the 11th the pulse was 96. The horse was not feeding, and further loss of condition had become evident. On the 12th the pulse was 96, the heart impulse was visible over a fairly large area, there was a slight expiratory dyspnoea present. On the 13th the negative venous pulse was strongly pronounced, the area of the heart

impulse was extended upwards and backwards. The nostrils were slightly distended, numerous small petechiae were seen on the septum nasi of the near nostril and on the plica sigmoidea. The horse was leaning forwards with legs spread apart, when walking it swayed and dragged its legs, it showed some whitewash marks on the skin of the supra-orbital processes. On the 14th June, 1917, the pulse was 116, the impulse was visible, extending backwards and upwards. Occasionally some contractions of the heart succeeding each other rapidly. The beating of the abdominal aorta was felt all along the backbone to the os sacrum. The negative venous pulse was strongly pronounced. Conjunctiva was ecchymosed and blotchy. The horse died on the 15th June, 1917.

Post-mortem.—Autopsy two and a half hours after death. Condition was poor. Abdomen was not distended. Rigor mortis was present. The integument was intact. Visible mucous membranes were somewhat pale. Pupilla was open. Anus closed. Subcutaneous tissue was somewhat ochre yellow. Only very little fat was present, which was ochre yellow in colour. The blood did not stain well, it was brownish. The flesh was brown and somewhat dry. Mandibular lymph glands were moist, not enlarged, the supporting tissue was oedematous, also the subparotid and retropharyngeal lymph glands. Upper cervicals were moist. Salivary glands: submaxillaries and parotids were slightly moist. Mucosa of tongue was covered with food. Near base of tongue was a big cicatrized fissure dividing the tongue into two. The follicles of the pharynx appeared as ink-black spots, the vessels were filled. The tonsillae were injected and raised, they had a mottled appearance. Ecchymoses were present on the caudal side of the epiglottis. Mucosa of larynx and trachea were smooth and glistening. The right thyroid was light brown, in the centre was a small white speck, fairly hard and raised. On left side behind the pharynx was a tumour, oval shaped, 14 × 9 cms., weighing 5 kilo., corresponding in position to the thyroid gland, its surface was smooth. On section a brown yellow liquid escaped. Some cysts were present, forming clefts, containing a viscid substance. The diaphragm had numerous fibrous filaments on both sides, but more on the peritoneal side. Peritoneal cavity: Only a few drops of liquid were present. In the regio hypochondrica of left side was an extensive suggilation, occupying a space of 30 × 10 cms.; it was situated behind the arcus costarum. On the peritoneum was present a large scar with a number of thick fibrous bands. The subperitoneal fat tissue on section appeared infiltrated with much blood. On the right side was a similar condition, but the peritoneum was smooth. Under the peritoneum of lumbar region was a haemorrhagic suffusion. The peritoneum was brownish, smooth, and glistening. The serosa of the intestines was the same. Diaphragm convexly forwards. Pleural cavities contained no liquid, number of small fibrous filaments were present on pleura of the heart region. Bronchial and mediastinal lymph glands were enlarged, moist, and black. Lungs were collapsed, pleura showed fibrous filaments. Extensive emphysema was present in both apices. Intermediary lobe of right lung was consolidated; on section pus was found in the bronchi. Left lung on section had pale appearance sprinkled with black anthracotic patches. The tissue was spongy. Mucosa of bronchus was smooth and glistening. Intima of artery was also smooth and glistening, the vein showed nothing unusual. In posterior portion of the artery was a small mixed thrombus, it branched into several vessels. Right lung was fairly rich in blood, it was slightly moist, the vein showed nothing unusual. The bronchus contained a little froth. Artery showed some thrombi in the posterior branches. At one place the thrombus was adherent to the intima, which was roughened; the lung at this place was rich in blood. Pericardium contained 20 c.c. clear brown liquid which had a tinge of green, otherwise nothing unusual was seen. Heart: left ventricle was in systole, right in diastole, and contained little non-coagulated blood. Epicardium was light brown, haemorrhagic effusions were present in the sulci longitudinales and ecchymoses and petechiae at the base where there was also a fair amount of fat. The right endocardium was light brown. In muscoli papillares of the septum were some superficial ecchymoses and also in all the valves. In the right anterior valve was a distinct suggilation. Endocardium of atrium showed ecchymoses. Semilunar valves showed nothing unusual. Left ventricle showed a small plasma coagulum under the valves. Endocardium was pale, ecchymoses were present in muscoli papillares and in septum. Bicuspid valves showed suggilations. The semilunars were normal. Intima of coronary arteries was smooth. Vena magna cordis showed nothing unusual. Left myocardium was 4, the right one 2 cms. thick, pale brown, opaque, resembling terra cotta, the left was slightly darker. Consistence was somewhat soft. Liver: the size was about normal, the capsule of

the posterior side was smooth, through it the liver parenchyma appeared mottled (nutmeg); some small points, 1 to 4 mm. in diameter raised and smooth were present; on anterior surface were fibrous filaments. Liver on section had a brownish green colour. Red streaks and points were noted in a yellow mesh-work. The consistence was fairly firm. The pancreas was moist, fairly rich in blood. Sclerostome larvae were found in it. Splenic lymph glands were somewhat moist and pale. Capsule of the spleen showed a few fibrous filaments and appeared shrivelled. The measurements were 48×27 cms. On section the pulp was dark brown, the follicles were not visible; trabeculae in parts were thickened, consistence was normal. The pulp could be scraped off. Right suprarenal glands were rich in blood, the cortex was thin in proportion to medulla. The left one was pale and moist on section. Kidneys: not much fat. Right capsule was somewhat adherent. Surface of the organ had a mottled appearance due to irregular bluish depressions filled with blood. On section the cortex was striated and had a pale yellow appearance. Consistence was rather tough. Also left kidney on surface showed irregular depressions. Parenchyma was pale. Cortex was somewhat opaque and slightly yellowish tinged, glomeruli were not visible. The stomach was distended, not much food was present. Fundus contained a mucous deposit, three spiroptera tumours were present, reaching the size of a nut. Mucosa of duodenum was bile stained, otherwise normal. Small intestines: the ileum was slate coloured and red patches were present on the mucosa, in some parts fairly extensively; mucosa of jejunum in parts slightly swollen. Large intestines: rectum contained some hard balls which were covered with mucus. The mucosa was red and covered with thick mucus. Caecum and colon: mucosa was grey with red patches in parts. Floating colon was covered with mucus. Mesenteric lymph glands were much enlarged, reaching the size of a hen's egg, they were embedded in watery tissue and had a reddish colour, and much blood escaped on section. The tissue showed oedematous infiltration. Some glands had a cystic appearance. Mesentery of colon and caecum showed suggillations. Intima of abdominal aorta was smooth and glistening. Anterior mesenteric artery showed nothing unusual. The bladder was empty, traces of brownish mucus were present. A little mucus was present in the urethra, the mucosa was smooth. The brain was pale and moist. Cerebellum showed nothing unusual. In femur and humerus was fat marrow with small haemorrhagic patches. First molar teeth on both sides showed large cavities. Body weight, 357.5 kg.; right lung, 5 kg.; left lung, 2.3 kg.; heart, 4.2 kg.; liver, 6 kg.; right kidney, .85 kg.; and left kidney, .75 kg. Liver: body weight=1: 59.

Pathological anatomical diagnosis.—Malnutrition. Anaemia. Icterus. Oedema of the peripharyngeal and perilaryngeal connective tissue of lymph and salivary glands. Cicatrix in tongue. Tonsillitis. Ecchymoses on epiglottis. Struma cystica. Haemorrhage in regio hypochondrica. Pleuritis fibroblastica. Anthracosis and oedema of bronchial lymph glands. Emphysema pulmonum. Pneumonia purulenta. Thrombosis of pulmonary artery. Ecchymoses in epicardiums and endocardiums. Fatty degeneration of myocardium. Fatty degeneration and pigmentation of the kidney. Sclerostome larvae in pancreas. Perisplenitis fibrosa. Fatty degeneration of kidneys. Spiroptera tumor in stomach. Hyperaemia of small and large intestines. Oedema of mesenteric lymph glands. Haemorrhages in mesentery.

Microscopical examination.—Liver, right lobe: the septa were slightly thickened, due to the presence of fibroblastic tissue and round cells and homogeneous substance between these elements. The lobules were fairly distinct, but many of the liver cells contained vacuoles and were out of shape and those around the central veins contained much pigment, so that brown tracts of liver cells were formed. The central veins in most parts were filled with blood, and also the adjacent capillaries. The lumina of the central veins contained in some places rather numerous leucocytes, and these were also scattered about between the liver cell rows. On a few places there was a proliferation of bile-ducts that entered into the periphery of the lobules. The Scharlach stain showed the presence of fat globules in the peripheral portion of the lobule. Middle lobe: here the newly formed bile-ducts appeared more numerous than in the previous section and formed in some places clusters interlocking with the liver cell rows, suggesting a replacement of the latter by the former. With the increase in the number of bile-ducts corresponded an increase of fibroblastic and fibrillar tissue of the septa. Right lobe: condition similar. In some places the septa showed no changes, whilst in others there was a conspicuous increase of round cells. Kidneys: some of the glomerulus capsules were thickened and some of the

glomeruli were atrophied. There was also a round cellular infiltration in the adventitia of some of the vessels. There was a fair amount of blood present. A number of tubuli contorti showed the presence of fat globules when stained with Scharlach and likewise the tubuli recti in the intermediary zone. Left kidney: some of the arteries showed hyaline degeneration of their walls. The fatty degeneration was distinct and more pronounced than in the right kidney. Heart, right wall: a considerable fatty degeneration was present unequal in intensity, so that a patchy appearance was produced visible by naked eye. At one place was an increase of fibroblastic tissue. Septum: the fatty degeneration was even more pronounced and of almost uniform intensity and patches of fibroblastic tissue were present irregularly outlined. The muscular fibres were running into and traversed them. Left wall: fairly extensive fatty degeneration and fibroblastic tissue formation was noted, the latter more conspicuous than in any of the two former sections. There were also patches in which the muscular fibres were broken to pieces, the fat globules scattered about and an increase of fibroblasts and round cells. Spleen: the blood sinuses contained much pigment, brown in colour, and of different grains. Some of the follicles appeared atrophied. Suprarenal glands: the cortex was diffusely red stained by Scharlach stain. Muscle: fatty degeneration of muscular fibres was present and a few of these fibres were wavy and telescoped. Thyroidea: the tumour was conspicuous by the absence of follicles containing colloidum, which were replaced by follicles without lumina. Lungs: the interlobular septa were thickened by oedematous infiltration. The bronchi were filled with polymorphonuclear cells, which in many parts showed karyorrhexis. In the adjacent alveoli was a homogeneous substance (oedema) containing desquamated cells.

Diagnosis.—Stasis, cirrhosis, atrophy and necrosis of liver cells. Pigmentation. Emigration of round cells. Regeneration of bile-ducts. Nephritis chronica and fatty degeneration. Myodegeneratio adiposa and myocarditis fibroblastica. Fatty degeneration of suprarenal glands and of muscles. Pigment of spleen and atrophy. Struma nodosa. Broncho-pneumonia crouposa.

Result.—This horse was not immunized against Horse-sickness; it consumed in one hundred and twenty-two days a total amount of 48 lb. Senecio. It died twenty days after discontinuation of feeding, having shown symptoms of illness for about a week preceding death. The symptoms of myodegeneration of the heart were well pronounced. On post-mortem the lesions of a parenchymatous Hepatitis, as well as those of an acute myocarditis, were found. This case is interesting since the disease developed after the feeding with Senecio had been discontinued, although it is true the interval was only a short one.

Experiment No. 19.—Horse 10755, an aged grey gelding, purchased and arrived at Onderstepoort in December, 1916. On the 23rd January, 1917, it was used in a Ragwort feeding experiment, receiving in its food 2 oz. of Ragwort daily up to the 29th, from the 30th January to the 7th February the quantity was increased up to 3 oz. daily, from the 8th to the 19th to 4 oz. daily, from the 20th February to the 3rd March to 6 oz. daily, when the feeding was stopped for a few days. From the 6th to the 27th it again received a daily dose of 6 oz., which was increased to 1 lb. daily from the 28th March to the 3rd April, after which it only received 6 oz. daily up to the 28th and 8 oz. daily from the 29th April to the 29th May, having eaten a total quantity of 50 lb. 3 oz. in one hundred and twenty-five days. This horse was not immunized. During the period of feeding, occasionally a disturbance in the temperature was noted with but slight exacerbations to 101° F. The morning temperatures kept at 99° F. and above. Three days before the discontinuation of the feeding, namely, on the 27th May, a fever exacerbation to 104° F. was noted in the evening, and the morning temperature was at 102.4° F. From the 14th June, 1917, a slight rise occurred, 101° F. in the morning and 102° F. in the evening. During this period, on the 17th, the mucous membranes of the eyes were found slightly congested, the pulse was 48; no further notice was taken of the animal until its death on the 19th June, 1917.

Post-mortem.—Condition fair, abdomen was distended. Rigor mortis was complete. Integument intact. Tongue was hanging out. Mucous membranes of mouth were brownish. Eyes were closed. Anus closed. Black, deeply stained blood flowed from axillary vein. Behind and under the shoulder of near side, below the musculus cutaneous were extensive suggilations. Flesh was dark. Mandibular lymph glands were rather small, retro-pharyngeals and subparotids were swollen and haemorrhagic and embedded in haemorrhagic tissue. Upper cervicals were somewhat moist. Both thyroids were dark in colour. Velum palatinum was dark in colour. Pharynx showed nothing unusual.

Vessels of the epiglottis were injected. Tongue showed a constriction at apex, otherwise nothing unusual. Oesophagus was somewhat bluish discoloured. Mucosa of trachea in lower portion showed some petechiae. Peritoneal cavity: serosa of intestines had a somewhat bluish appearance. The parietal peritoneum was bluish grey in colour. Large intestines and stomach were distended with gas. Diaphragm convexly forwards, haemorrhagic suggillations and ecchymoses were present on posterior surface. On anterior surface were fibrous filaments. Pleural cavities: in mediastinum were extensive suggillations along the dorsum. They were also present but not so extensively in the posterior mediastinum. Bronchial and mediastinal glands showed haemorrhagic infiltrations. Left lung was half collapsed. Extensive emphysema was present. Rest of pleura was somewhat blue. Diaphragmatic pleura showed fibrous filaments and a few ecchymoses. In right lung extensive emphysema was present in anterior and main lobes. Pleura was smooth. Left lung on section was dark, it contained black blood. Its consistence was elastic. The vein showed nothing unusual. The bronchus contained froth, its mucosa was diffusely reddened and numerous ecchymoses were present. Artery showed nothing unusual. The right lung was rich in blood, the tissue was elastic. The vein showed nothing unusual, the bronchus contained froth and numerous petechiae on the mucosa. Lung showed some oedema. Intima of thoracic aorta was smooth and glistening. Mucosa of oesophagus showed nothing unusual. There was froth in the trachea, and a few ecchymoses on the mucosa. Upper cervical lymph glands were enlarged, moist, black, haemorrhagic, the supporting tissue was oedematous. Pericardium: in the walls were traces of fat of a red ochre colour and ecchymoses were present at the base of the aorta. The sac contained 130 c.c. of clear dark brown liquid. Serosa parietalis was smooth and glistening. Heart: right ventricle was empty, the ostium was open for a hand. Left was also empty, it was slightly contracted. The epicardium showed in sulci longitudinales and at base extensive suggillations and haemorrhages, very marked on margo caudalis of left ventricle. Right endocardium was pale brown, few petechiae were on the wall, on septum, and musculus papillaris cranialis. Semi-lunar and tricuspid valves showed nothing unusual. In left endocardium were found extensive suggillations on septum and muscoli papillares, semi-lunar valves were normal. Bicuspid valves were slightly ecchymosed. Intima of coronary arteries was normal. Vena magna cordis showed a suggillation under the intima corresponding to that on outside. Myocardium had a greyish dirty opaque appearance: left wall 4 cms., right 2 cms. thick. Diaphragmatic surface of liver showed three fibrous patches and some filaments. Visceral surface was smooth. Lymphatic vessels stood out prominently. The edges were fairly sharp. Colour was light brown with some small yellow patches. On section parenchyma had a light yellow appearance. Lobulation was not very distinct. Lobules appeared yellow. The periphery appeared white and was slightly sunken. The central vein was not prominent. The consistence was firm. The central veins in the right lobe were distended. Veins in middle lobe, in places also distended. Increase of interlobular connective tissue was marked, but not extensive. Some stasis was present in the right lobe. Splenic lymph glands were normal. Spleen, 56×25 cms. Capsule appeared normal. On section the pulp appeared black. Trabeculae were distinct. Follicles not distinct. Consistence showed nothing unusual. Left suprarenal gland was fairly rich and the right very rich in blood. Kidneys: left showed some fat, capsule was easily detached. The surface of the organ showed nothing unusual. On section medulla was noted pale. Intermediary zone was diffusely injected. Cortex had a light yellowish appearance. Glomeruli were visible. Consistence showed nothing unusual. Right capsule was easily detached. The surface was light brown and smooth. On section similar condition to left was noted, in some places the yellow discoloration was more pronounced than in others. Stomach contents were liquid and soft food. Fundus was slightly slate coloured. A spiroptera tumour was present. In cardiac portion numerous young gastrophilus larvae were present. Pylorus showed nothing unusual. The duodenum was slightly discoloured. Mucosa of jejunum and ileum slightly slate coloured. Caecum and colon: mucosa slate coloured. Rectum mucosa somewhat bluish discoloured. Mesenteric lymph glands were somewhat swollen and moist. On section liquid escaped. Some oedematous infiltration was present in the mesentery. Abdominal aorta showed nothing unusual. In arteria ileo-caeco-colica an aneurisma was found. The bladder was empty. Mucosa showed extensive ecchymoses. Brain: the vessels of the pia were injected. The substance was somewhat moist. Bone marrow of femur was pure fat, that of the humerus contained fat with a small red focus. Teeth were irregular, first and third molars on upper side were long and on lower side correspondingly depressed.

Pathological anatomical diagnosis.—Subcutaneous haemorrhage behind shoulder. Stasis and oedema pulmonum. Pleuritis fibrosa. Icterus. Emphysema pulmonum. Ecchymoses in mediastinum, on pleura, and in lymph glands, pericardium, epicardium, and endocardiums. Stasis, pigmentation, and cirrhosis of liver. Stasis in lymph vessels of Glisson's capsule. Haemorrhages in diaphragm, fatty degeneration of kidneys. Fatty degeneration of heart. Tumor splenis. Cicatrix in tongue. Hyperaemia of intestinal mucosa. Oedema of mesentery and lymph glands.

Microscopical examination.—Liver, right: there was a thickening of the septa due to the formation of fibroblasts and fibrillar connective tissue and to round cells, these elements being somewhat separated by an intervening homogeneous substance. In the septa and in the periphery of the septa were newly formed bile-ducts, most of which were radiating into the periphery of the lobules. The course of these bile-ducts was usually not a straight one, but twisted, and between them a fair amount of fibrillar connective tissue was present. In the middle zone and the centre of the lobule were remnants of liver cell rows between which there was a transparent homogeneous substance. The liver cells themselves were out of shape and disconnected; narrow, polygonal, and bizarre forms were present. Their staining was dark and in conspicuous contrast to the lighter coloured new bile-ducts. The central veins were filled with blood, which also filled the capillaries leading into them. There were a good number of leucocytes scattered about. There was also some light yellow brown pigment present and much of it in the connective tissue supporting the newly formed bile-ducts. Scharlach stain showed the fat to be principally in the periphery of the lobules. Also some of the newly formed bile-ducts showed fat globules, finely distributed in the epithelial cells. Middle and left lobe showed identical conditions. Kidney, right: the greatest number of tubuli contorti showed absence of nuclei, and in some the epithelial cells were desquamated and the nuclei pyknotic. Pycnosis was also present in some of the tubuli recti. At some places in the adventitia of vessels were small round cell conglomerations. A few of the glomeruli were atrophied or undergoing atrophy. The Scharlach stain showed in a portion of the tubuli contorti the presence of fat distributed in fine granules; it was also present in some of the tubuli recti of the intermediate zone. Left kidney: the conditions were similar to those of the right one, some capsules were thickened. The Scharlach stain showed the thin portions of Henle's loop to contain fat in the epithelial lining. Heart, right wall: the fibres of the greater portion of the section showed diffuse infiltration with fat globules, so much so that the Scharlach-stained section macroscopically appeared red. There were patches in which the fibres were absent or only portions of such present, the deficiency being made up by fibroblastic tissue rich in round cells. Septum: similar conditions. Left wall: there were patches in which the muscular fibres were broken to pieces and scattered between the pieces were large cells and round cells and in some parts homogeneous substance. In other parts the fibroblastic tissue was well pronounced, it included portions of fatty degenerated fibres. Between the fibres in some parts a slight increase of leucocytes was noted. Suprarenal glands: diffuse fatty infiltration of the cortex, so that the Scharlach-stained section macroscopically appeared red. Muscle: some of the fibres showed diffuse fat staining with Scharlach and the fibres so affected were wavy in their course. Spleen: there were some dark grains of pigment in the sinuses and a fair amount of red corpuscles.

Diagnosis.—Atrophy and fatty degeneration of liver cells and regeneration of bile-ducts; emigration of round cells, oedema. Fatty degeneration of kidneys and necrobiosis of epithelial cells; atrophy of glomeruli. Myocarditis fibroblastica and fatty degeneration. Fatty degeneration of suprarenal glands. Fatty degeneration of skeletal musculature. Pigment in spleen and hyperaemia.

Result.—This horse was not immune against Horse-sickness. During a period of one hundred and twenty-five days it consumed a total amount of 50 lb. Ragwort. The horse died twenty-three days after discontinuation of feeding. The post-mortem showed the lesions of Ragwort poisoning, viz., acute parenchymatous Hepatitis and Myocarditis. The regeneration of bile-ducts was prominent. The interesting feature in this case is the observation, that the animal died of typical Ragwort poisoning after the feeding was discontinued.

Experiment No. 20.—Horse 10766, an aged grey gelding, purchased and arrived at Onderstepoort in December, 1916. On the 23rd January, 1917, it was placed in a Ragwort feeding experiment, receiving 2 oz. daily until the 29th, the quantity was increased to 3 oz. daily from the 30th January to the 7th February, to 4 oz. daily from the 8th to the 19th, to 6 oz. daily from the 20th February to the 3rd March, when the feeding was stopped for a few

days. From the 6th to the 27th March the horse received 6 oz. daily, from the 28th March to the 3rd April 1 lb. daily, and from the 4th April to the 28th April 6 oz., and from the 29th April to the 29th May 8 oz. daily, consuming in all 50 lb. 3 oz. in one hundred and twenty-five days. This horse was not immunized against Horse-sickness. During the feeding experiment occasionally a slight exacerbation of temperature was noticed, usually it kept at 99° F. in the morning and rarely went over 100° F. in the afternoon. On the 2nd July, 1917, the horse showed a sharp rise from normal in the morning to 104.6° F. in the afternoon. On the 4th the temperature returned to 101° F. in the morning and 101.4° F. in the afternoon and kept at this figure until the 7th, when another rise to 103° F. took place, succeeded two days later by a remission of 100° F. Subsequently the temperature dropped and the horse died on the 11th July. On the 3rd July, 1917, the pulse was 88, the impulse was not much pronounced. The horse when standing showed a peculiar rocking movement, backwards and forwards, it shifted its hind legs frequently and kept its head hanging, the eyes were half closed, the nostrils were slightly open; slight dyspnoea was present, when walking a slight swaying was noticed; the conjunctiva had a washed-out appearance. On the 4th July, 1917, the pulse was at 84. The negative venous pulse was well pronounced and a loss of condition had become noticeable. On the 5th the horse was found down in the morning. The urine collected was yellowish and clear, bile pigments were distinctly present, albumen amounted to .05 per cent. On the 6th similar conditions as on previous day, only a very small amount of urine was passed during the night, which contained .05 per cent. albumen. On the 8th the pulse was 84, the impulse extended, the horse was losing condition rapidly and appeared to be weak in the loins. On the 9th the horse was seen to have been down overnight, but the condition was similar to previous day. On the 10th the pulse was 96 and very small. The respirations numbered 8, the inspirations were long drawn, and in the expirations there was a double movement of the flanks, stopping short suddenly, almost with a jerk. On the 11th July the pulse was imperceptible, the impulse not strong, the horse was frequently down, it still showed a long drawn inspiration and a short expiration. It died the same day.

Post-mortem.—Autopsy was made soon after death. The condition was rather poor. The abdomen was sunken. Rigor mortis was not present. Integument of head showed some abrasions on the processus condyloideus of near side. The pupillae were open. Visible mucous membranes were pale. Anus was closed. The blood was not coagulated, pale and not staining well, somewhat watery, and had a peculiar brownish hue. Flesh was somewhat pale and dry in appearance. The traces of fat had a yellowish hue. On dorsum of tongue was some thick deposit which could be scraped off. In the wall of the left guttural pouch was a haemorrhage, the size of a sixpence, with a necrotic centre. Velum palatinum showed nothing unusual. In the follicles of the tonsilla were several small pieces of grass. Salivary glands showed nothing unusual. Sub-mandibular lymph glands were rather small, the retro-pharyngeals and upper cervicels showed nothing unusual. The parotids were pale. In the pharynx was found one *gastrophilus* larva and some mucus. The mucosa was pale. The oesophagus showed nothing unusual, the trachea was pale. On opening the peritoneal cavity some yellow liquid escaped. A considerable amount of fat was under the peritoneum of the flanks. Situs viscerum was normal. The serosa was pale. The peritoneum parietale glistening. The omentum showed the presence of some fat. Diaphragm was convexly forwards. No foreign contents present in the pleural cavities. Costal pleura was smooth, somewhat yellowish tinged. Bronchial and mediastinal lymph glands were anthracotic, not enlarged. Right lung was adherent by a fibrous connection to pleura in region of seventh rib. Both lungs were inflated. Pleura of right lung showed numerous ecchymoses. Emphysema was present in both apices. Left lung on section was poor in blood. Parenchyma was elastic. Mucosa of bronchi and intima of vein and artery showed nothing unusual. More blood was found in right lung, the parenchyma was elastic. The artery, vein, and bronchus showed nothing unusual. Mucosa of thoracic trachea was normal. Mucosa of oesophagus was normal, some food particles were present, and in lower portion two larvae (*gastrophilus pecorum*). Intima of aorta and of truncus brachiocephalicus smooth. Lower cervical and anterior mediastinal lymph glands were enlarged, haemorrhagic, and embedded in oedematous tissue. Posterior side of diaphragm, both in tendinous and muscular portions, showed extensive haemorrhagic infiltrations and fibrous filaments. Pericardium showed some fat under the pleural lining and contained 60 c.c. clear brownish liquid. The parietal serosa was smooth and glistening. Both ventricles were empty, the right was

flabby, in diastole, the atrium was empty. Ostium open to hand. Left ostium was tight for four fingers. Good amount of fat was present in the sulci. Epicardium showed nothing unusual. Left endocardium was slightly pale, a gelatinous infiltration was present and a few ecchymoses on septum. Bicuspid valve showed some suffusion. Semi-lunar valves showed nothing unusual. The right endocardium was pale and slightly yellowish tinged. One of the valves showed a haemorrhagic infiltration. Arteriae coronariae showed nothing unusual. Intima of vena magna cordis smooth. Foramen ovale was closed. Left myocardium was 4 cm., the right 2 cm., thick, of a pale greyish opaque appearance and patchy due to irregular darker areas. Consistence was soft, somewhat like putty. Periportal lymph glands were enlarged and moist. Liver appeared enlarged, margins were slightly blunt. The colour was reddish brown. The lymphatic vessels of the caudal surface were very distinct. The capsule was smooth. On cranial surface were fibrous filaments. Right lobe on section was fairly rich in blood, it had a granite like appearance due to yellow and red coloured patches. The lobulation was not quite distinct. The central veins seen in places were surrounded by a greyish zone and further by a reddish zone. The consistence was firm, not tough. Middle lobe and left lobe were similar, here the whitish meshes were more prominent. Pancreas was rich in blood, moist, otherwise normal. The splenic lymph glands were infiltrated with blood. Spleen measured 45×21 cm., the capsule was smooth. Pulp was brownish. Trabeculae were just visible, the follicles were not visible. Consistence was firm. Suprarenal glands: Cortex was rich in fat. Kidneys: good amount of fat was present in the capsule. Right kidney on section was light brownish in colour and the various zones were not well marked. The glomeruli were just visible. The consistence was about normal. The parenchyma of the left kidney was fairly rich in blood. The cortex was yellowish tinged. The glomeruli were just visible. The consistence was somewhat soft. The stomach contained but little food. A dry blackish deposit was present in the fundus, the mucosa showed nothing unusual. Gastrophilus larvae were on cardiac portion, a small spiroptera tumour was present. In the duodenum was a yellowish deposit. Some ascaris were present, mucosa showed nothing unusual. Mucosa of ileum and jejunum in parts swollen, in parts reddened, and in parts bile stained. Mucosa of caecum and colon thickened, in parts reddened, and even haemorrhagic. Mucosa of floating colon was slightly thickened and diffusely reddened. Mucosa of rectum was covered with mucous deposit, otherwise it appeared normal. Mesenteric glands were enlarged, haemorrhagic. Intima of mesenteric and abdominal aorta showed nothing unusual. Arteria ileo-caeco-colica showed slight roughening of the intima. Bladder contained turbid brownish urine. The vessels of the mucosa were injected. Brain was pale, the ventricles were of usual size. In femur yellow marrow was surrounded by red marrow. The humerus marrow was yellow with a red focus. Body weight, 311 kg.; right lung, 3.2 kg.; left lung, 1.8 kg.; heart, 2.9 kg.; liver, 10.1 kg.; spleen, 1.5 kg.; right kidney, .9 kg.; and left kidney, .9 kg. Liver: body weight=1: 30.7.

Pathological anatomical diagnosis.—Traumatism in skin. Anaemia. Pleuritis fibrosa. Emphysema pulmonum. Icterus. Fatty degeneration of myocardium. Parenchymatous degeneration of liver. Perihepatitis fibrosa. Fatty degeneration of kidneys. Tumour splenis. Gastritis. Enteritis catarrhalis et haemorrhagica. Necrosis in guttural pouch. Slight ascites. Anthracosis of lymph glands. Gastrophilus larvae in pharynx and oesophagus. Endoarteritis of arteria ileo-caeco-colica.

Microscopical examination.—Liver, right: there was a slight thickening of the septa mainly due to the presence of round cells, between which in some parts some fibrillar connective tissue was visible. Adjacent to the septum was a zone of liver cells, the majority containing vacuoles. The capillaries around the central veins were confluent and the cells themselves reduced in size, distorted and darker stained, and contained pigment. In the intermediate zone vacuolated liver cells were seen amongst the blood corpuscles. There was no change in the walls of the central veins. In some places between the capillaries a slight increase of leucocytes was noted. The Scharlach stain showed a corona of fatty cells around the centre of the lobule, which was occupied totally by red corpuscles, or there was a radiating reticulum between the meshes of which the corpuscles were found. The fat globules were of different sizes. Middle lobe: the central portions of the lobules were filled with blood forming small pools, in some lobules almost reaching the periphery. In the Scharlach-stained section the liver cells contained fat globules, so that a green central portion containing

blood corpuscles was surrounded by a red ring of fat globules. There was also some light yellow brown pigment present, and it was mingled with the fat cells or bordering the septum. In some of the relatively intact cells green pigment (bile) was seen filling the bile-ducts between the cells and thus forming a reticulum. There was no change in the central vein. Left lobe: in this section the bile pigment was particularly conspicuous in the form of engorged bile-ducts between the liver cells and in the form of flakes, so that the tissue in some parts had a greenish tinge. In some places the fat globules were very large, larger than a liver cell, and apparently formed by the coalescence of two or more drops. Also yellow pigment was present. Kidney, left: in some places around the vessels were round cells and at one place the lumen of the vessel itself was crammed full. Some of the glomeruli were atrophied, being small and contracted, some were hyaline with only a few pycnotic nuclei left within. Some of the glomeruli capsules were slightly thickened. The Scharlach stain showed the presence of fat globules in the epithelial cells, principally along the outer contour of the cells. The fat globules were small, only occasionally some were of larger size. The tubuli contorti contained much fat, it was present to a much smaller degree in the tubuli recti. In the intermediate zone a good amount of fine fat globules were found in the thinner tubules of Henle. Right: in some tubules the cells were desquamated and the nuclei pycnotic. At one place a streak of connective tissue was radially placed with a vessel within. Otherwise similar conditions were found as in left kidney. Heart, left: the Scharlach-stained cross-section had a patchy appearance due to the staining of bundles or portions of bundles of fibres, so that macroscopically fatty degeneration was evident. Septum: here were patches in which the fibres were absent and replaced by a fibroblastic tissue rich in nuclei, there was a destruction of fibres in some places and a collection of leucocytes. These cells were also scattered throughout the section, in some places more than in others, filling in some parts the clefts between the fibres. The Scharlach-stained section showed macroscopically a patchy appearance. These places corresponded with those of cellular infiltration mentioned above. The fibres were crammed full of finely divided fat globules. Right: under the endocardium a good portion of fibroblastic connective tissue was present, occupying the place of the former fibres, and round cell infiltration was mingled with it. The Purkinje fibres, between the endocardium and the fibrous organization, were vacuolated, and the Scharlach showed the presence of large fat drops. This stain also showed some of the fibroblasts to contain fat droplets. There was a fairly diffuse red staining of the fibres, more intense in some places than in others, and also some of Purkinje fibres within the myocardium showed the presence of fat. Spleen: a small amount of blackish grains of pigment was present in the sinuses, which contained but little blood in some parts and a fair amount in others. Lymph glands: There was blood in the sinuses and mingled with it dark brown pigment; in some places the germinative follicles were entirely surrounded by blood. A vein in a septum contained a considerable number of leucocytes. In one of the glands the lymphoid tissue was reduced to small clusters, most of which were triangular shaped. The sinuses were filled with large pale cells mingled with a few blood cells. In some places a homogeneous substance was present in these sinuses. Skeletal muscle: quite a fair number of fibres took the Scharlach stain and quite a number of these were broken, the broken pieces showed fat stain. In some places pieces were broken out, and such broken pieces no longer showed any structure. Suprarenal glands: macroscopically the Scharlach-stained section showed diffuse red coloration.

Diagnosis.—Liver: Stasis, slight cirrhosis. Round cell emigration, fatty degeneration, and atrophy of liver cells. Icterus and pigmentation. Myocarditis fibroblastica et myodegeneratio adiposa. Fatty degeneration of kidneys. Pigment in spleen. Lymphadenitis catarrhalis et haemorrhagica. Fatty and waxy degeneration of the skeletal muscle. Fatty degeneration of the suprarenal glands.

Result.—This horse was not immunized against Horse-sickness. During a period of one hundred and twenty-five days it consumed 50 lb. of Senecio. It died forty-three days after feeding of the plant had been discontinued. Symptoms of illness some days before death were well pronounced. The lesions found were those of an acute Hepatitis, in this case without the formation of new bile-ducts. Inflammatory and degenerative processes were present in the heart and fatty degeneration of the various parenchymatous organs. This case is interesting, since the disease developed forty-three days after discontinuation of feeding the poisonous plant.

Experiment No. 21.—Horse 10768, an aged bay gelding, purchased and arrived at Onderstepoort in December, 1916. On the 23rd January, 1917, it

was put into a Ragwort feeding experiment, receiving daily 2 oz. until the 29th, when the dose was increased to 3 oz. daily from the 30th January, 1917, to the 7th February, to 4 oz. daily from the 8th to the 19th, to 6 oz. from the 20th February, 1917, to the 3rd March. The feeding was stopped for a few days, and began again on the 6th March with 6 oz. daily until the 27th, from the 28th March, 1917, to the 3rd April 1 lb. daily, from the 4th to the 28th 6 oz. daily, and from the 29th to the 26th May 8 oz. daily, a total amount of 48 lb. 11 oz. in one hundred and twenty-two days. During the period of feeding the temperature kept normal as a rule, there was an occasional slight exacerbation to 102° F., but there was no definite curve present. On the 29th May, 1917, the horse was visibly ill, it had not been feeding well for the last two days and had rapidly lost condition, the temperature curve during the last eight days had showed a disturbance with an occasional exacerbation to 102° F. The pulse on the 29th May was 56, the negative venous pulse was well pronounced, the impulse of the heart was increased in intensity, the sounds were somewhat confluent. The conjunctiva had a slight yellowish tinge. When walking a swaying gait was noticed. On the afternoon of the same day the horse was down in its box and kept in a sternal position. In the evening it was found stretched out breathing heavily. It died during the night. The examination of the blood was negative.

Post-mortem.—Condition was rather poor. Rigor mortis was present. The integument showed nothing unusual. Conjunctiva was slightly oedematous and yellowish. Subcutaneous tissue had a saffron colour. Flesh slightly yellowish. Salivary glands were slightly yellowish. Mandibular lymph glands were slightly yellow and oedematous. Thyroids showed nothing unusual. Muscles of the tongue were slightly yellowish. Oesophagus showed nothing unusual. Trachea contained foam, it was yellowish, and the mucosa showed slightly diffuse hyperaemia. Larynx and pharynx were similar. Fibrous filaments were present on posterior surface of diaphragm. Right lung was slightly enlarged. On section it showed hyperaemia and oedema. Intima of pulmonary artery was slightly yellowish. In bronchi was a small amount of foam, mucosa was slightly yellowish and diffusely hyperaemic. Left lung was normal in size, the pleura was yellowish. On section hyperaemia and oedema were noted. Intima of pulmonary artery was yellowish. Bronchi contained foam, they were yellowish, and mucosa showed diffuse hyperaemia. Thoracic trachea contained foam and mucus and mucosa showed diffuse hyperaemia. The oesophagus showed nothing unusual. Intima of aorta was yellowish. Pericardium contained 300 c.c. turbid brownish liquid. Epicardium was slightly yellowish, fairly numerous small ecchymoses were present at base. Right endocardium showed a big ecchymosis at base of bicuspid valve and numerous smaller ecchymoses and petechiae. Left ventricle contained dark coagulated blood. Endocardium showed numerous haemorrhagic extravasations. Myocardium was yellowish, it was softer than usual. Liver contained numerous fibrous filaments on caudal surface. On section parenchyma had a nutmeg appearance. Small whitish soft foci were within. Centres of lobules were brown, peripheral parts were yellowish. Consistence of the organ was fairly tough. Pancreas had a slight saffron colour. Splenic lymph glands were enlarged and oedematous. Spleen measured 40 × 20 cm. On section it had a dark colour, trabeculae and follicles were not distinct. Consistence was soft. Suprarenal glands were enlarged. On section medulla was found hyperaemic, cortex was yellowish. Kidneys: Boundary zone was slightly hyperaemic. Cortex was brown yellow in colour. Fundus of stomach was diffusely hyperaemic, blood coagula were present mixed with mucus, on the mucosa were petechiae. Cardiac portion showed numerous gastrophilus larvae. Mucosa of jejunum showed haemorrhagic extravasations in patches, ileum ditto, also diffuse hyperaemia was present. Part of ileum showed a slate colour. On mucosa of the apex of caecum were large patches of haemorrhagic extravasation, a diffuse hyperaemia and oedematous infiltration of the submucosa was noted. In the colon were large patches of haemorrhagic extravasation also oedematous infiltration of the submucosa. Mucosa of rectum showed patchy hyperaemia. Mesenteric lymph glands were enlarged and oedematous. Mesentery showed diffuse hyperaemia. Intima of abdominal aorta was yellowish. In the branches of the mesenteric artery was a thrombus and some nematode larvae. Bladder was distended by clear dark brown urine, mucosa showed numerous petechiae, and had a slight saffron discoloration. Urine examination: NH_3 - $\text{N} = .017 = 5$ per cent. of total in protein nitrogen. Amino acids: Trace only. Albumen 4/1000 or 0.4 per cent. Haemoglobin and methaemoglobin were marked. Bile also was present. Brain: pia mater was slightly yellowish. Substance on section showed a very slight yellowish discoloration. Bone marrow of humerus was

gelatinous, saffron in colour; that of the femur was likewise gelatinous with haemorrhagic infiltration and of saffron discoloration. Teeth: upper molars showed marked decay. Body weight, 260 kg.; right lung, 5.7 kg.; left lung, 4.4 kg.; heart, 3 kg.; liver, 4.15 kg.; spleen, 1.7 kg.; and kidney, 1.9 kg. Liver: body weight=1:62.6.

Pathological anatomical diagnosis.—Icterus. Slight hydropericardium, hyperaemia, and oedema of lungs; fatty degeneration of myocardium; ecchymosed epicardium and endocardiums. Tumour of the spleen; haemorrhagic gastritis, haemorrhagic enteritis; petechiae in bladder; parasitic thrombosis; stasis and fatty degeneration of liver. Perihepatitis fibrosa. Haemoglobinuria. Caries of upper molars. Gastrophilus larvae in stomach.

Microscopical examination.—Liver: the section had a somewhat greenish aspect, and there was a meshwork present of irregularly arranged greenish meshes interlaced with irregularly outlined whitish meshes. Under lower power it could be seen that the contours of the green meshes were shading off. The white meshes belonged to the periphery of the lobule and included the veins which were cut crossways and longitudinally, standing out prominently, and filled with blood. Within the greenish bands were the central veins somewhat hidden. Under higher power the periphery of the lobules appeared translucent and devoid of cells. There was no distinct increase of connective tissue, no eosine tinged fibres, what was present appeared to be left over after the liver cells had partially or completely disappeared. Such cell remnants could frequently be seen as empty structures with a nucleus, the cytoplasm being vacuolated entirely or in parts. These cells were not the old liver cells, but apparently new ones, to judge by their pale colour, and in some parts by the arrangement of their nuclei which indicated small bile-ducts or appeared as multinuclear or giant cells. In the Scharlach-stained section these cells stood out very prominently and showed intensive fatty degeneration, likewise did the cells lining the old bile-ducts within the septa. At one place these newly formed bile-ducts were very numerous, penetrating into the peripheral portion of the lobule and replacing the old liver cells, which by their different colour (brown) could easily be recognized. The central vein showed a slight thickening. A somewhat hyaline band was formed by the adventitia and between the lumen and the adventitia was found a loose structure of long spindle cells, embedded in a transparent substance. In some parts it was distinctly fibrillar and within red corpuscles were scattered about, the lumen was occupied by red corpuscles which obscured the presence of an epithelial lining. A similar structure was also found in some places of the septa, indicating here a new formation of fibrillar connective tissue. The capillaries running into the central veins contained much blood, the course of the veins was indicated by the presence of this blood, distending in part the capillaries fairly wide and forming small blood-pools. The adjacent liver cells had disappeared or were reduced in size, showed different shapes and were vacuolated. Many contained pigment, this was green in some cells, when it could be seen that the small bile-ducts between the cells were plugged with bile or it was light brown and granular. The pigment was also found in the cells of the intermediary zone, showing various forms, being irregularly arranged and containing vacuoles. There was in some parts of the intermediate zone and adjoining the central vein an increased number of nuclei of the round cell type. Kidney: some of the glomeruli capsules were thickened. In the tubuli contorti some of the nuclei were pycnotic. At some places were small round cell collections near a vessel. There was a considerable amount of blood present in the intermediary zone. Scharlach stain showed the presence of fat globules in the majority of the tubuli contorti and also in the tubuli recti. Suprarenal glands showed diffuse staining with Scharlach so that the section was red. Heart: fairly diffuse staining with Scharlach of the fibres to a fairly severe degree in some places. Muscle: fairly diffuse staining of quite a number of fibres with Scharlach. Spleen: a fair amount of blood and pigment was in the sinuses. Lungs: the bronchi were filled with desquamated cells. The capillaries were distended with red corpuscles and the lumen of the alveoli was reduced, containing homogeneous substance and desquamated cells. Also blood was present in some of the alveoli.

Diagnosis.—Liver: Slight central and peripheral cirrhosis; stasis, fatty degeneration; icterus; emigration of leucocytes; regeneration of bile-ducts and liver cells. Fatty degeneration of kidneys, suprarenal glands, heart, and muscle. Pigment in kidneys. Bronchitis and oedema pulmonum.

Result.—This horse had received a total amount of 48 lb. 11 oz. of Ragwort in one hundred and twenty-two days and died of Ragwort poisoning four days after discontinuation of feeding. Characteristic lesions were found in the liver,

which can all be summarized under the name of Hepatitis parenchymatosa and cirrhosis. The latter may or may not be a sequel. It may have been present before, since such conditions are commonly found in South African horses and in particular in those suffering from the disease *Dunziëkte*, that may be present unexpectedly in any horse. The description of the histological changes also resembles somewhat the picture given as acute Liver-atrophy. There is only a difference in degree. The destruction of the liver cells had not advanced so far and a fair amount of newly developed liver parenchyma was present that apparently replaced the former tissue.

Experiment No. 22.—Horse 10774, an aged grey gelding, purchased and arrived at Onderstepoort on the 4th December, 1916. On the 23rd January, 1917, it was put into a Ragwort experiment, receiving a daily dose of 2 oz. until the 29th, from the 30th January, 1917, to the 7th February 3 oz. daily, from the 8th to the 19th 4 oz. daily, from the 20th February, 1917, to the 3rd March 6 oz. daily. The feeding was discontinued for a few days, but recommenced on the 6th March when the horse received 6 oz. daily until the 27th, 1 lb. daily from the 28th March, 1917, to the 3rd April, 6 oz. from the 4th to the 28th, and 8 oz. daily from the 29th April to the 29th May, having consumed 50 lb. 3 oz. in one hundred and twenty-five days. The temperature of this horse during the period of feeding was fairly normal, occasionally it showed some disturbances but not of much consequence. On the 12th July, 1917, namely, forty-three days after the discontinuation of feeding, the horse was immunized against Horse-sickness with Tzaneen virus. It passed through a mild reaction. Thereafter the horse lost condition and was killed on the 10th August.

Post-mortem.—Autopsy was made half an hour after death. The condition was fair. The abdomen was not distended. Rigor mortis was not present. The visible mucous membranes were pale. The natural openings were normal. The flesh was dark in colour. Subcutaneous tissue contained a fair amount of fat. The blood was not coagulated and dark in colour. Sublingual and submaxillary glands were slightly hyperaemic. One parotid showed a melanoma in upper half (about size of hen's egg). Submandibular lymph glands were normal. The pharyngeals contained some small melanotic tumours. Upper cervicals were slightly hyperaemic. Both thyroids were dark in colour and small in size. The left thyroid showed a strumous cyst. Muscles of the tongue were normal. Oesophagus and pharynx were normal. Larynx and trachea showed nothing unusual, as did the peritoneal and pleural cavities. Bronchial and mediastinal lymph glands showed anthracosis. Left lung was collapsed. Pleura was smooth. Lung on section showed a slight hyperaemia. Consistence was elastic. The bronchus was normal. The artery contained partly coagulated blood. The right lung was collapsed, the pleura was smooth. Lung on section showed stasis, the consistence was firm. The bronchus was normal, the artery contained partially coagulated blood. The thoracic trachea was normal, also the oesophagus and aorta. Pericardium contained 100 c.c. reddish yellow turbid fluid. Circulatory organs: right ventricle and atrium contained partially coagulated blood. The ostium was open for the hand. Left ventricle and atrium were empty, ostium open for four fingers. The epicardium was normal. Right endocardium and valves showed nothing unusual. Left endocardium and valves were also normal. Myocardium, left 4, right 1.5 cm. thick. On section somewhat pale in colour. The consistence was firm. Liver capsule was shrunken and showed fibrous filaments. In parenchyma were numerous small white calcareous nodules, size about a pin's head. On section it was pale in colour. The lobulation was distinct. Consistence was firm. Centres of lobules were dark brown and the peripheries were pale in colour and enlarged. The pancreas was normal. Splenic lymph glands showed nothing unusual. Spleen, 43 × 18 cm. Capsule was normal. On section some trabeculae were seen. Colour and consistence of parenchyma were normal. Spleen showed a small accessory portion, about the size of a pigeon's egg, with an apparently normal spleen structure. Right and left suprarenal glands were normal. The capsules of the kidneys were easily detached. The glomeruli were distinct. The colour was dark. The consistence firm. Fundus of the stomach showed *gastrophilus* larvae, both equi and pecorum. A spiroptera tumour was present, about the size of a hen's egg. Mucosa of duodenum, of small intestines, colon, floating colon, and rectum normal. Intima of abdominal aorta was normal. In the arteria ileo-caeco-colica was a small aneurism. The bladder contained some turbid yellow urine. Skeleton: ankylosis of right front fetlock joint, foot being bent at right angle to metacarpal bone. Bone marrow of humerus showed a slight and that of femur a marked haemorrhagic infiltration. Teeth were normal. Body weight, 281.5 kg.; right lung, 2.18 kg.; left lung, 1.96 kg.; heart, 2.46 kg.; liver, 3.7 kg.; spleen, 1.23 kg.; and kidneys, 1 kg. Liver: body weight=1:76.

Pathological anatomical diagnosis.—Slight hydropericardium; slight hyperaemia of left lung; stasis of right lung. Atrophy of liver. parasitic nodules in liver. Fatty degeneration of myocardium. Atrophy of both thyroids. Melanoma. Struma nodosa. Anthracosis. Spiroptera tumour. Gastrophilus larvae in stomach. Ankylosis of fetlock. Anaemia.

Microscopical examination.—Liver, right lobe: the liver structure was normal. The liver cells, however, appeared somewhat patchy due to different shades, some parts of the cells being transparent. This transparency was more pronounced in some lobules than in others. There was also occasionally a vacuole present, a round hole sharply cut out. There was no increase of interlobular connective tissue, the portal veins were filled with blood, only in a few places was there any blood in the central vein. The nuclei present were of different sizes and shading, in transparent cells there was generally also a pale nucleus. In the Scharlach-stained section fat globules were seen corresponding to the well defined vacuoles. There was some light brown yellow pigment present in the liver cells adjacent to the central vein. There was a similar shading of the liver cells as in the Haemotoxylene-eosine stained section. Also the endothelial cells contained small fat granules. Occasionally in a septum or between the liver cell rows abutting on to a septum was a small collection of round cells. In a large round nucleus of liver cells some fat granules were seen, the nucleus being stained rather deeply. There were also parasitic nodules present. Left lobe: the presence of pigment was well pronounced around the central veins. Otherwise there were identical conditions as described above. Middle lobe: same condition. Kidney: there were a number of glomeruli in the process of atrophy. Spleen: there was a considerable amount of dark pigment in the blood sinuses, which were moderately filled with red corpuscles. In some places appeared to be an atrophy of the lymph follicles; the arteries were seen surrounded with only a small number of lymph cells and the follicles were crowded in the particular portion. Some of the arteries in a follicle appeared to undergo hyaline degeneration. Suprarenal glands: the cells of the zona fasciculata immediately adjoining those of the zona glomerulosa were white transparent. There was some bright yellow brown pigment in the cells of the zona glomerulosa. The vessels were fairly well engorged. Scharlach stain gave a well pronounced fat reaction and in particular of the light coloured zone described above, the cells of which were uniformly filled. Thyroids: there was some dark brown pigment present in some of the follicles, it was situated in the cells and also in the lumen. Muscle: no abnormalities were seen.

Diagnosis.—Very slight fatty degeneration of the liver. Atrophy of the glomeruli of the kidney. Fatty degeneration of the suprarenal glands. Pigmentation of the thyroids

Result.—This horse received in one hundred and twenty-five days a total amount of 50 lb. 3 oz. of Ragwort. No symptoms were noted during this period and none after. The fact that the horse passed through a mild Horse-sickness reaction did not cause the evolution of parenchymatous Hepatitis. The horse lost in condition steadily and was killed seventy-three days after discontinuation of feeding. On post-mortem no lesions of a parenchymatous Hepatitis were present. There was, however, a difference in the shading of the liver cells, which might have been due to regenerative processes, so that in this instance a recovery might have taken place.

Experiment No. 23.—Horse 10890, an aged bay gelding, purchased, arrived at Onderstepoort on the 15th December, 1916. On the 23rd January, 1917, it was placed in a Ragwort feeding experiment, receiving daily 2 oz. until the 29th, from the 30th January, 1917, to the 7th February 3 oz. daily, from the 8th to the 19th 4 oz. daily, from the 20th February, 1917, to the 3rd March 6 oz. daily, when the feeding was stopped for a few days. From the 6th to the 27th it again received 6 oz. daily, from the 28th March to the 3rd April 1 lb. daily, from the 4th to the 28th 6 oz. daily, and from the 29th April to the 29th May 8 oz. daily. A total amount of 50 lb. 3 oz was consumed in one hundred and twenty-five days. During the period of feeding the temperature of this horse had generally a normal course, only occasionally an exacerbation, never passing 102° F., was noticed. On the 12th July, 1917, forty-three days after the feeding had been discontinued, this horse was immunized against Horse-sickness with Tzaneen virus for the first injection, and a mixture of Tzaneen, Onderstepoort, and Bulawayo viruses for the second injection with serum "O" on the 7th and 9th day. The horse showed a typical Horse-sickness reaction and developed a Dikkop from which it died on the 12th day after the first injection; the symptoms previous to death were all those of the Dikkop type.

Post-mortem.—The autopsy was made one hour after death. The condition was fair, the abdomen was normal. Rigor mortis was present. Integument showed abrasions on temporal region. Natural openings showed nothing unusual, pupillae were dilated. Visible mucous membranes of the eyes were markedly pale. Bulging of both temporal fossae was present. Flesh was slightly moist, rather dark in colour, gelatinous infiltration of connective tissue was noted in region of neck and axilla. Blood was not coagulated, it was very dark in colour, slightly thick in consistence and stained deeply. The salivary glands were slightly hyperaemic, of a somewhat mottled appearance, reddish and grey on section. Thyroids and mandibular lymph glands were normal, also tongue, oesophagus, and pharynx. Trachea vessels were slightly injected. The larynx contained a small quantity of mucus, mucosa was normal. Peritoneal cavity showed nothing unusual. A few fibrous filaments were present on both sides of the diaphragm. Pleural cavities: numerous ecchymoses were seen on pleura on both sides and on the mediastinum. Bronchial lymph glands were slightly enlarged and oedematous, as were also the mediastinal lymph glands. Left lung was partly collapsed. Some patches of petechiae were on dorsal border. Parenchyma on section showed a well marked hyperaemia and slight oedema. Artery and vein showed nothing unusual. Mucosa of bronchus showed numerous petechiae and the vessels were somewhat injected. Lung tissue was fairly elastic. The right lung was the same as the left one. Thoracic aorta and oesophagus showed nothing unusual. Trachea vessels were injected. Prepectoral lymph glands were slightly hyperaemic and oedematous. Pericardium contained 1255 c.c. dark yellow, slightly turbid fluid. Both ventricles were empty. Right ostium admitted the fist, left only four fingers. Epicardium showed very numerous petechiae and ecchymoses on the ventricular wall and along the coronary furrows. In the right ventricle was a large bluish black extravasation on endocardium of sides and septum. In left ventricle the extravasations were even more marked. On both sides these extravasations extended under the valves. Left myocardium 3.5, right 1.5 cm. in thickness, they were grey in colour. Consistence was somewhat softer and colour more opaque than usual. Liver: some fibrous filaments were present on anterior surface. Right lobe was very much smaller than left. The edges of right lobe were fairly sharp, also left edge of middle lobe. Lower edge of middle lobe was more rounded. Through the capsule the lobulation could be seen mapped out by yellowish grey lines, the colour inside the meshwork being reddish brown. On section left lobe cut firmly. Lobulation was distinct. Tissue between lobules was slightly contracted, of a greyish colour, and slightly translucent. Centres of lobules reddish. Periphery was yellowish. Consistence of parenchyma was firm. Pancreas showed nothing unusual, neither did the splenic lymph glands. Spleen measured 48 by 25 cm. Pulp was firm, moist, slightly swollen. Trabeculae were not distinct. Follicles were slightly enlarged. Left and right suprarenal glands were slightly hyperaemic and slightly enlarged. Capsule of left kidney was very slightly adherent. The cortex was pale brown and opaque, the vessels appeared injected, forming vertical red lines. Medullary and boundary zones were dark reddish in colour, due to the injection of vessels. Consistence showed nothing unusual. The right kidney was similar in appearance to left. Stomach: mucosa showed a marked diffuse discoloration, varying from reddish, through a dark red to a somewhat bluish red colour. Numerous gastrophilus larvae were present. Mucosa of small intestines was thickened and markedly oedematous, somewhat wrinkled and yellowish in colour. Mucosa of caecum and colon thickened and markedly oedematous. Restum showed a patchy hyperaemia towards the anus. Mesenteric lymph glands were pinkish in colour. In the anterior mesenteric artery was nothing unusual. The bladder contained a small quantity of turbid yellow urine. Mucosa was somewhat reddish in colour. Urethra showed nothing unusual. Brain showed no abnormalities. The bone marrow of the femur showed red foci, that of the humerus was fat throughout. Teeth: the anterior edge of the first lower left molar was somewhat projecting and a corresponding wearing on upper tooth was present. The posterior upper left molars were worn below the level of the gum. The teeth sloped backwards and upwards. Body weight, 367.7 kg.; right lung, 2.55 kg.; left lung 3.08 kg.; heart, 3.25 kg.; liver, 4.42 kg.; spleen, 1.98 kg.; and kidneys, 1.6 kg. Liver: body weight=1: 83.

Pathological anatomical diagnosis.—Hydropericardium, ecchymoses, petechiae and extravasations in epicardium, extravasations under both endocardiums. Ecchymoses on pleurae. Hyperaemia and slight oedema of lungs. Slight tumour splenis. Cloudy swellings of parenchyma of heart and kidneys. Interstitial Hepatitis and fatty degeneration. Hyperaemia of gastric mucosa.

Oedema of intestinal mucosa, oedematous and hyperaemic lymph glands. Oedema of subcutaneous connective tissue of neck, axilla, and chest. Gastrophilus larvae in stomach.

Etiological diagnosis.—Horse-sickness.

Microscopical examination.—Liver: the lobulation was quite distinct. In parts every lobule was isolated by a thin septum surrounding it completely, in others only partially. In the centre a number of cells showed rather large vacuoles and with the Scharlach stain large fat droplets, the central vein and the adjacent capillaries being filled with red corpuscles. Some of the septa were slightly thickened by connective tissue, the fibrillar bundles and round cells showing up well, the latter not being very frequent. The striking feature was the presence of light coloured cells, which in the septa proved to be bile-ducts and liver cells adjacent to the periphery of the lobule. There they interlocked with the liver cell rows, and continued the row of the darker stained cells, thus completing them. Sometimes also in the middle of a lobule and in a row between older liver cells were the lighter ones to be seen. Alongside of them and detached were small groups of round cellular accumulations. Kidney, right: at some places near the course of an artery and at one place directly under the capsule were small foci of round cells, fairly densely packed. Within the focus under the capsule the tubules were either missing or atrophied. The Scharlach stain showed fat in the tubules of the intermediate zone. Left: same condition as in right one. Some of the tubuli contorti showed also fat globules. Right heart: in some places the muscular fibres had disappeared and in their place was a transparent connective tissue, fairly rich in spindle shaped narrow nuclei, whilst in some places the number of round cells had increased. The Scharlach stain showed only a few of the fibres to possess fat, and only in a moderate degree. In the left wall the fatty patches were more numerous but by no means frequent. Suprarenal glands: Scharlach showed a patchy staining, some patches more intensively stained than others, and amongst them were patches not stained at all. Muscle: some of the fibres showed fat reaction. They were not frequent.

Diagnosis.—Atrophy, necrosis, and fatty degeneration of liver cells, regeneration of bile-ducts and liver cells; slight cirrhosis; emigration of round cells. Nephritis interstitialis chronica. Myocarditis fibroblastic and myodegeneratio adiposa. Fatty degeneration of kidneys and suprarenal glands.

Result.—This horse consumed during a period of one hundred and twenty-five days a total amount of 50 lb. of Ragwort. It was a non-immunized horse, and forty-three days after discontinuation of feeding was placed in a Horse-sickness experiment and died twelve days after the injection of the first virus from Dikkop. The lesions found on post-mortem were those of Horse-sickness. The microscopic examination of the liver showed the presence of a parenchymatous Hepatitis, the regenerative processes were pronounced as well as the fatty degeneration in other organs. This case is interesting as the cause of death was apparently not the Ragwort poison but Horse-sickness, and on post-mortem the typical lesions of Ragwort poisoning were found. It is possible that the horse would have succumbed to the disease subsequently. It showed that forty-three days after discontinuation of feeding lesions of Ragwort poisoning can still be found.

Experiment No. 24.—Horse 10896, an aged brown mare, purchased and arrived at Onderstepoort on the 15th December, 1916. The horse was placed in a Ragwort feeding experiment on the 23rd January, 1917, receiving daily 2 oz. until the 29th, 3 oz. daily from the 30th January, 1917, to the 7th February, 4 oz. daily from the 8th to the 19th, 6 oz. daily from the 20th February, 1917, to the 3rd March, when the feeding was stopped for a few days, but recommenced on the 6th with 6 oz. daily until the 27th, 1 lb. daily from the 28th March, 1917, to the 3rd April, 6 oz. daily from the 4th to the 28th, and 8 oz. daily from the 29th April to the 26th May, consuming in all 48 lb. 11 oz. in one hundred and twenty-two days. The temperature of this horse during the feeding period was fairly normal, occasionally some exacerbations were noticed passing 102°, but no definite curve could be detected. On the 12th July, 1917, forty-five days after discontinuation of the feeding experiment, this horse was immunized against Horse-sickness, Tzaneen virus being used followed by serum on the 7th and 9th days, it passed through a distinct reaction without any further symptoms. The horse rapidly fell off in condition after the 12th July, 1917. It was killed on the 4th August, 1917, on account of malnutrition.

Post-mortem.—Autopsy was made half an hour after death. Abdomen was sunken. Condition was poor. Rigor mortis was absent. Integument was intact. Pupillae were open. Some blood was found around the nostrils (bullet).

There was no fat in the subcutaneous tissue. The fasciae were whitish. The blood was dark brown red, not coagulated, and did not stain well. Muscles had a brownish hue. Pharynx contained a few *gastrophilus* larvae near the base of tongue. Larynx contained some blood and mucus, mucosa was pale. Mucosa of guttural pouches was smooth. Submaxillary and parotid glands showed no abnormalities. Retropharyngeal lymph glands were somewhat enlarged and white. Submandibulars were distinct and nodules well separated. Upper cervicals were slightly enlarged and pale. Haemorrhagic infiltration was present in subcutaneous tissue around the pharynx (bullet wound). Right thyroid was light brown, containing whitish foci, the largest one was in the centre. The left thyroid showed a similar focus in the periphery. A scar was near the apex of the tongue and another somewhat higher cutting off a tumour like portion. The oesophagus was normal. Serosa of peritoneum and intestines was pale. Situs viscerum was normal. Diaphragm convexly forwards, fibrous filaments were present on posterior side. A little brownish liquid was present in pleural cavities. Left lung was attached to costal pleura between the fifth and seventh ribs. Otherwise pleura costalis smooth and glistening. Lungs were collapsed. Bronchial and mediastinal lymph glands were anthracotic. Extensive emphysema was present in apices of both lungs and extending along the ventral border. On pleura pulmonalis were present some white patches and filaments, otherwise it was smooth and glistening. Parenchyma of left lung on section was somewhat pale and bloodless. Consistence was elastic; the bronchus contained some froth, the artery a coagulum, and lower down a white thrombus; the vein was normal. Right lung contained a little more blood, it was moist on section. The bronchus contained some froth and mucus. Artery showed a coagulum and some thrombotic substance, the vein showed nothing unusual. Thoracic trachea contained a little mucus. Oesophagus showed nothing unusual. Intima of aorta and truncus brachio-cephalicus normal. Anterior mediastinal and lower cervical lymph glands appeared somewhat enlarged and moist; the supporting connective tissue was slightly oedematous. Pericardium contained a small quantity of brownish liquid. Serosa of parietal portion was smooth and glistening. The right ventricle contained some uncoagulated blood, which did not stain well and was brownish. Ostium admitted a hand. The left ventricle and atrium were empty. Some oedematous fat was found in the sulci. The epicardium was smooth and glistening, also the left endocardium. Under the endocardium of left musculus papillaris was a suffusion of blood and one under the anterior bicuspid valve. Semilunar valves showed nothing unusual. Endocardium of atrium was slightly yellowish tinged. Septum of right ventricle slightly yellowish, smooth, and glistening. In the septum of the atrium a suffusion was present. Foramen ovale was closed. Vena magna and coronary arteries were normal. Myocardium was pale brown, left 4.5, right 2 cm. thick. Consistence was normal. Periportal lymph glands were somewhat pale and moist. Right lobe of liver was reduced in size, 10×12 cm. Left lobe appeared thickened. Fibrous filaments were present on anterior surface. Posterior surface was smooth, some of the lymphatic vessels stood out distinctly. Through the capsule could be recognized a number of bluish irregularly shaped foci, on section they were noted entering a little distance in to the parenchyma. The parenchyma had a dark brownish appearance. Darkish foci, as seen under the capsule, were present throughout the substance. With a hand lens a brownish discoloration of the lobular centres and a greyish discoloration of the peripheries were seen. Some of the septa appeared thickened. Consistence was fairly firm. Middle and right lobes showed similar structures. Pancreas: colour was light brown. An aneurism, a thrombus, and some nematode larvae were present in one of its arteries. Splenic lymph glands showed nothing unusual. Spleen measured 36×17 cm. Some fibrous filaments were present on capsule of the caput. Lymph vessels in hilus were distended. Pulp was light brown on section, trabeculae were distinct, follicles not visible. Left and right suprarenal glands were poor in fat, also left kidney; the capsule was easily detached. Surface of the organ in parts somewhat irregularly contracted. On section the three zones were distinct. Glomeruli were distinct. Intermediary zone was fairly dark. Consistence was fairly firm. The region of the superficial contractions showed on section whitish areas. Right kidney capsule was the same as the left. On section of the organ the intermediary zone was distinct, it was injected. The glomeruli were not so distinct as in the left kidney. Consistence was about normal. The stomach contained a little coarse food. On mucosa was a mucous deposit. Some *gastrophilus* larvae were on cardiac mucosa. A small spiroptera tumour was present. Mucosa of duodenum was thickened, yellowish. Mucosa of small intestines was slightly thickened, it appeared oedematous and much folded and

yellowish discoloured. Rectum contained balls of faeces. The mucosa was slightly pale. Mucosa of caecum and colon thickened and pale. Mesenteric lymph glands showed no abnormalities. The mesentery was poor in fat. Intima of abdominal aorta was normal. In anterior mesenteric artery and ileo-caecocolica was some thrombotic substance, a large aneurism with much thickened walls was present in the latter. Sexual organs: the uterus was distended with liquid. On opening, a white substance escaped, fairly thick and cream like. Mucosa was bluish red. No offensive smell was present. Vagina was slightly pale and yellowish discoloured. Ovaries were much enlarged, irregular in outline, on section white, and contained a number of small cysts. Brain at base was haemorrhagic and contained some blood in ventricles (bullet wound) otherwise normal. Teeth were very irregular. The last molar of mandibula on right side was loose and could be pulled out. The alveolar cavity was filled with food stuffs. The second molar was outwardly directed and the third inwardly. Upper teeth were also very irregular, the molar teeth on the right side being longer. The femur contained some yellow marrow with red border along the posterior substantia comparta, the marrow of the humerus contained a small haemorrhagic focus in its lower end. Body weight, 279 kg.; right lung, 2.3 kg.; left lung, 2 kg.; heart, 2.8 kg.; liver, 3.3 kg.; spleen, .7 kg.; left kidney, .55 kg.; and right kidney, .6 kg. Liver: body weight=1 : 84.5.

Pathological anatomical diagnosis.—Anaemia. Marasmus. Pyometra. Thrombosis in lungs. Aplasia of right liver. Oedema of lymph glands. Parenchymatous degeneration of liver. Perihepatitis fibrosa. Fibrous infarcts in kidney. *Gastrophilus* larvae in pharynx and stomach. Struma nodosa. Cicatrix in tongue. Parasitic aneurism and thrombosis in mesenteric artery.

Microscopical examination.—Liver, right lobe: the conspicuous feature in this section was the presence of budding bile-ducts in and along the septa without any or at least no noticeable increase of connective tissue. In most places these bile-ducts interlocked with the liver cell rows, which they apparently replaced. There were a certain number of round cells present. Light brown pigment was present in the cells around the central veins. The Scharlach stain showed the presence of fat in only one portion of the section and here in the middle zone of the lobules. There was also dark brown pigment present in some of the liver cells of the periphery. Middle lobe: here the conditions were identical, but in some parts the accumulation of round cells was very conspicuous in the septa and a considerable amount of bright yellow brown pigment was present, the majority of cells containing it, some lobules having quite a yellowish aspect; only occasionally a patch of fatty cells was seen. Kidneys: a few of the glomeruli were atrophied. There was in some places a round cellular infiltration in the adventitia of a vessel. No fat was noted. The wedge shaped patches seen by naked eye penetrating the cortex from the periphery consisted of a reticulum, the contents of the meshes having fallen out, so that it had somewhat the appearance of a piece of emphysematous lung. In some of the meshes was a pinkish substance resembling colloid. Many of the meshes seemed to be lined with a flattened epithelium, whilst others seemed to be devoid of it. In the central portion of this area was a patch of round cells, all placed around a small forking vessel replacing the tubular tissue. Spleen: some brown yellow pigment was present in the sinuses. Heart: there was a slight fatty degeneration present and of uniform distribution in all the fibres of both the walls and the septum. Suprarenal glands: there was a moderate amount of fat present in the cortex and particularly in the zona glomerulosa. Thyroids: in one section were a number of small dense foci, from one to four millimetres in diameter. In these foci the colloidal follicles were absent or only scantily present and scattered about. The rest was occupied by follicles without any contents or only in a reduced quantity. There was also some pigment in the epithelial wall of the normal portion. In a second section such a focus was about 1 cm. in diameter. The greater portion of follicles was devoid of colloid. The same section contained also some smaller foci. In one of them, the connective tissue septa were arranged somewhat in a rootstock fashion, branching from a centre into different directions. In a third section in place of colloidal substance in some of the follicles was a white transparent substance. The follicles in the neighbourhood of all these foci were compressed and formed oblong clefts, which in some parts were very narrow.

Diagnosis.—Slight fatty degeneration of liver; emigration of round cells; regeneration of bile-ducts; pigment. Slight myodegeneratio adiposa cordis. Pigment in spleen. Cystic kidney. Struma nodosa.

Result.—This horse consumed during a period of one hundred and twenty-two days a total amount of 48 lb. of Ragwort. Forty-five days after discontinuation of feeding the horse was immunized against Horse-sickness. From this date onwards it rapidly lost condition and was killed on the seventieth day after discontinuation of feeding, being then very poor in condition. The interesting feature was the presence of a parenchymatous Hepatitis with the regeneration of the bile-ducts well pronounced. The loss of condition necessitated the killing of the horse, and this wasting was probably a symptom of the disease which might, however, also find an explanation in the presence of the pyometra. The symptoms of fatty degeneration were also pronounced in some of the parenchymatous organs.

Experiment No. 25.—Horse 10907, an aged brown gelding, purchased and arrived at Onderstepoort on the 15th December, 1916. On the 23rd January, 1917, it was placed in a Ragwort feeding experiment, receiving 2 oz. daily until the 29th, 3 oz. daily from the 30th January to the 7th February, 4 oz. daily from the 8th to the 19th, 6 oz. daily from the 20th February to the 3rd March, when the feeding was stopped for a few days, it again received 6 oz. daily from the 6th to the 11th when the feeding was stopped altogether, the horse having consumed 12 lb. 5 oz. in forty-six days. This horse showed previous to feeding normal temperatures, which were maintained during the period of Ragwort feeding. On the 27th February, 1917, whilst the horse was still in the feeding experiment, however, a temperature curve developed going over into a continuous fever and the presence of a pneumonia was diagnosed of which the horse died; the symptoms of the pneumonia were of a gangrenous nature.

Post-mortem.—Autopsy was made half an hour after death. Condition was fair. The abdomen was not distended. Rigor mortis was absent. The integument was normal. Natural openings and visible mucous membranes were normal. The muscular and subcutaneous tissues were normal. Blood exuding from the cut surface was dark and tar like; it coagulated rather slowly. The tongue was normal. The parotid and submaxillary glands showed nothing unusual. The bronchial and mediastinal lymph nodes were much swollen, oedematous, and haemorrhagic. The mandibular lymph nodes were normal. The retropharyngeal lymph nodes were slightly injected. The thyroids were normal. The pharynx was normal. The trachea contained a small amount of foam. The vessels of the oesophagus were slightly injected. The left jugular vein contained some coagulated blood. The vessels of the thoracic trachea near the bifurcation were injected, some muco-purulent fluid was present. Peritoneal cavity: a slight excess of dark fluid was noted. A few fibrous filaments were present on parietal peritoneum. A few filaria were seen. Diaphragm: on anterior surface were fibrous adhesions along the muscular border and filaments over the whole surface; acute hyperaemia was present and ecchymoses along the lower border. On caudal surface were some fibrous filaments. The left pleural cavity contained 100 c.c. blood-stained liquid, fibrous adhesions were present in the region of the fourth and fifth ribs. Over the first twelve ribs the parietal pleura was turbid, injected, and full of ecchymoses. In the mediastinum the blood-vessels were engorged and standing out distinctly. A focus of necrotic tissue was present. The right pleural cavity contained 100 c.c. blood-stained liquid. Blood-vessels of parietal pleura were very distinct, and a red fibrinous deposit covered the whole surface. Left lung was not collapsed. Pleura of lobus apicalis and lower half of corpus pulmonis showed a dark reddish colour with haemorrhagic infiltration, on diaphragmatic surface were fibrous filaments. The bronchus contained slightly blood-tinged foam. Pulmonary veins contained coagulated blood, the intima was normal, also that of the artery. On section the parenchyma of the corpus pulmonis was seen to be haemorrhagic in patches and purulent foci were present. About in the middle of the lung the parenchyma of the dependent portion was partly consolidated and contained numerous necrotic areas, varying from a pin's head to a bean in size, greyish in colour, and irregular in outline. These areas were slightly protruding. Lobus apicalis showed consolidation with necrotic abscesses and nodules from a pin point to size of a hen's egg. Right lung: the pleural vessels were injected. A patch 15x4 cm. of coagulated exudate was on the diaphragmatic surface. Ecchymoses and a protruding nodule (size of hazel nut and caseous) were present. The lung was not collapsed. Pericardium contained 50 c.c. brownish coloured fluid. In the right ventricle was a large blood clot, the atrium was distended with a blood clot and some dark fluid blood. Ostium was tight for a fist. In the left ventricle was a clot and some uncoagulated blood. The atrium was distended with a clot. Ostium was open for four fingers. Blood-vessels of the epicardium were injected and showed diffuse haemorrhagic infiltration on right

side, particularly along the sulci. Myocardium: right 1.8 and left 4 cm. thick and light greyish brown in colour. Atrium: intima pale, tricuspid valves were normal. Right endocardium was pinkish in colour, capillaries were injected, and a few ecchymoses were present on septal surface. Periportal lymph nodes were haemorrhagic and oedematous. The liver was swollen, dark in colour. Capsule on anterior surface of right lobe showed two greyish yellow areas near renal extremity with a thickening. Fibrous filaments were present. On posterior surface were also found a few fibrous filaments. Lower border of left lobe was very sharp with shrinking of parenchyma. The upper edge was more rounded, it was rich in blood. The parenchyma protruding on section. The colour was pale greyish brown, the consistence was soft, lobulation not distinct. Middle lobe: the lobulation was more distinct and the borders were normal. Lower borders of right lobe were same as left. The parenchyma was richer in blood. Pancreas had a normal appearance. The splenic lymph nodes were enlarged and haemorrhagic. The spleen was of normal size, 39×18 cm. The capsule showed small ecchymoses along the base of outer surface. The inner surface was the same, the pulp was normal, the trabeculae were distinct, follicles were not very distinct. Suprarenal glands: left, swollen, slightly oedematous, pale in colour; the right was of normal size, the cortex was injected. Right kidney: the fibrous capsule was adherent; organ on section was rich in blood; cortex and medulla were hyperaemic. Left capsule also somewhat adherent, but stripped more easily than the right one, except over a patch, 10×5 cm., where the capsule was thickened and adherent to the substance covering some purulent foci, resembling those of the lungs in size from a pea to a five shilling piece and surrounded by a zone of haemorrhagic infiltration. On section the posterior half of the organ was hyperaemic and showed purulent foci scattered throughout the substance, the majority were the size of a pea. Stomach: mucosa of villous portion was slightly congested, duodenum also. Mucosa of jejunum and ileum was apparently normal. Large intestines: mucous membranes showed patchy congestion, some nematodes were present. Small colon: a mucous coagulum was found adherent to surface of mucosa. Rectum contained a few balls of faeces coated with mucus. Mesenteric glands: swollen and haemorrhagic. Abdominal aorta and mesenteric artery showed nothing unusual. Bladder contained a litre of muddy coloured urine. The vessels of the mucosa were injected. The brain showed a haemorrhage, caused by the bullet. Bone marrow of femur and humerus showed no abnormalities, the teeth were in good condition. The nasal cavity showed no abnormality. Body weight, 299.6 kg.; heart, 2.9 kg.; liver, 6.75 kg.; spleen, .85 kg.; and kidneys, 2.5 kg. Liver: body weight = 1:44.3.

Pathological anatomical diagnosis.—Necrotic pneumonia of both lungs, pleuritis, ecchymoses on epicardium. Nephritis apostematosa. Fatty degeneration and congestion of liver. Peritonitis fibrosa.

Microscopical examination.—Lungs: the alveoli were plugged with homogeneous substance (serum) or with leucocytes or both. The septa were thickened by homogeneous infiltration. The bronchi contained plugs of pus cells and their coats were infiltrated with pus cells. In some places larger areas showed almost exclusively leucocytes (pus cells) in the alveoli and usually abutting on to a bronchiolus. There were foci of necrosis surrounded by pus infiltration of the surrounding alveoli, the polymorphonuclear cells adjacent to the necrotic centre showed strong pycnosis and a demarcation wall was formed, somewhat interrupted by the septa of the alveoli which showed up as clear spaces. Liver: the greater portion of the section showed a normal liver picture, at one place a diffuse stasis was present, the capillaries being distended and the liver cell rows reduced to narrow bands. A thick septum entered here into the section, consisting mainly of fibrillar connective tissue, some of the septa in the neighbouring area were also thickened in a somewhat similar manner. Kidney: in the region of the intermediary zone were irregularly shaped foci of necrosis and pus. Some of these foci were surrounded by round cells of lymphocytic and polyblastic type. The glomeruli showed a thickening of the capsule due to some homogeneous substance, separating the walls from the adjacent tubules; this substance was also separating the tubules in the close vicinity. Some glomeruli showed capsules thickened by connective tissue and some were obliterated.

Diagnosis.—Broncho-pneumonia suppurativa mortificans. Stasis and cirrhosis in liver. Atrophy of glomeruli of the kidney.

Result.—This horse contracted a pneumonia and was killed. The interesting point that concerns us here is the fact that no changes were noted in the liver due to feeding with Senecio, although the horse had consumed 12 lb. spread over forty-five days. The animal was killed five days after feeding was stopped.

Exp. No.	Animal No.	Date of Commencing.	Place.	Quantity Fed.	Days Fed.	Average per day.	Result.
1	H. 5192	1.11.11	Mooi River...	104 lb. 5 oz.	84	19.8 oz.	Died of Senecio poisoning during experiment.
2	H. 3772	1.11.11	Mooi River...	Dose in paddock for 3 days.	61	34 days.	Discharged.
3	H. 3779	1.11.11	Mooi River...	13 lb. 4 oz.	—	3.9 oz.	Discharged.
4	H. 5042	9.1.12	Mooi River...	8 lb. 4 oz.	—	—	Discharged.
5	H. 4529	9.1.12	Mooi River...	8 lb. 4 oz.	—	—	Discharged.
6	H. 6152	9.1.12	Mooi River...	8 lb. 4 oz.	—	—	Discharged.
7	H. 8220	27.9.13	Onderstepoort.	130 lb. 0 oz.	78	26.6 oz.	Showed staggers and recovered 16 days after discontinuation of feeding during a Horse-sickness reaction.
8	H. 8225	27.9.13	Onderstepoort.	132 lb. 0 oz.	73	26.7 oz.	Died of Horse-sickness 13 days after discontinuation of feeding and on post-mortem lesions of Senecio poisoning.
9	H. 9451	11.11.15	Onderstepoort.	48 lb. 4 oz.	44	17.5 oz.	Died of Senecio poisoning during experiment.
10	H. 8912	14.12.15	Onderstepoort.	17 lb. 11 oz.	80	3.5 oz.	Died of Horse-sickness during Senecio feeding experiment; on post-mortem, lesions of Senecio poisoning.
11	M. 9425	14.12.15	Onderstepoort.	43 lb. 3 oz.	114	6.0 oz.	Killed 3 days after discontinuation of feeding; no lesions of Senecio poisoning.
12	H. 10394	28.9.16	Kokstad....	31 lb. 14 oz.	50	19.2 oz.	Killed 3 days after discontinuation of feeding whilst showing symptoms of Senecio poisoning.
13	H. 9614	28.9.16	Kokstad....	44 lb. 10 oz.	58	12.3 oz.	Still alive.
14	H. 9200	16.3.16	Onderstepoort.	27 lb. 0 oz.	43	10.0 oz.	Not diseased; died in Arsenic experiment.
15	H. 3751	20.3.16	Onderstepoort.	24 lb. 0 oz.	39	9.8 oz.	Not diseased; died in Arsenic experiment.
16	H. 7293	23.1.16	Onderstepoort.	39 lb. 4 oz.	103	6.0 oz.	Died of Horse-sickness 23 days after discontinuation of feeding; on post-mortem, lesions of Senecio poisoning.
17	H. 7299	23.1.16	Onderstepoort.	39 lb. 3 oz.	103	6.0 oz.	Died of Senecio poisoning 96 days after discontinuation of feeding.
18	H. 10704	23.1.16	Onderstepoort.	48 lb. 11 oz.	122	6.3 oz.	Died of Senecio poisoning 20 days after discontinuation of feeding.
19	H. 10755	23.1.16	Onderstepoort.	50 lb. 3 oz.	125	6.4 oz.	Died of Senecio poisoning 23 days after discontinuation of feeding.
20	H. 10763	23.1.16	Onderstepoort.	50 lb. 3 oz.	125	6.0 oz.	Died of Senecio poisoning 43 days after discontinuation of feeding.
21	H. 10768	23.1.17	Onderstepoort.	48 lb. 11 oz.	122	6.3 oz.	Died of Senecio poisoning 4 days after discontinuation of feeding.
22	H. 10773	23.1.17	Onderstepoort.	50 lb. 3 oz.	125	6.4 oz.	Killed 73 days after discontinuation of feeding; no lesions of Senecio poisoning present.
23	H. 10774	23.1.17	Onderstepoort.	50 lb. 3 oz.	125	6.4 oz.	Died of Horse-sickness 56 days after discontinuation of feeding; lesions of Senecio poisoning present.
24	H. 10896	23.1.17	Onderstepoort.	48 lb. 11 oz.	122	6.3 oz.	Killed 70 days after discontinuation of feeding; lesions of Senecio poisoning present.
25	H. 10907	23.1.17	Onderstepoort.	12 lb. 5 oz.	46	4.2 oz.	Killed 5 days after discontinuation of feeding; no lesions of Senecio poisoning present. Pneumonia.

APPENDIX III.

EXPERIMENTS TO TRANSMIT ACUTE LIVER-ATROPHY (STAGGERS) BY INFUSION OR INJECTION OF BLOOD OR SERUM OR EMULSION OF LIVER TISSUE OF HORSES THAT SUFFERED OR DIED FROM THE DISEASE.

I. *Experiment*.—Infusion of Staggers blood into horses 10954 and 10955.

Horse 10209.—(History of this horse *vide* Appendix I.)—On the 27th February, 1917, during an attack of acute Staggers, blood of this horse was infused into two susceptible horses 10954 and 10955 for a period of ten minutes, corresponding approximately to a quantity of five litres per animal.

(1) Horse 10954, an aged chestnut gelding in good condition, arrived in Onderstepoort on the 21st December, 1916, and since this date was kept on daily temperature; its record remained normal until the time the infusion took place, i.e. on the 27th February, 1917 (5 litres). From the 5th day after the infusion the evening temperature rose, and a regular ascending curve ensued, morning and evening temperature each succeeding day gradually rising, until the evening of the 13th day. The horse died during the following night of Horse-sickness. The symptoms of this disease became evident on the 12th day in the form of acute dyspnoea (Dunkop). The blood of horse 10209 thus still contained the virus of Horse-sickness, viz., 66 days after the first or 58 days after the second injection of Horse-sickness virus.

(2) Horse 10955, an aged bay gelding in good condition, arrived in Onderstepoort on the 21st December, 1916, and since that date was kept on daily temperature, with no abnormal curve during this period. Infused on the 27th February, 1917 (5 litres). The horse was kept under further observation until the 12th July, 1917, again with a normal temperature record and a normal health during this period. On this date the horse was inoculated for Horse-sickness, receiving 5 c.c. virus of horse 11357 Tzaneen, and on the 7th and 9th day respectively 165 c.c. and 245 c.c. serum 1715. A typical Horse-sickness reaction developed, the fever curve lasting from the 5th to the 13th day. The symptoms of Dikkop started on the 13th and lasted to the 21st day. On the 18th September, 1917, the horse was submitted to virus "O." immunization, receiving 5 c.c. virus of horse 10920 intrajugularly simultaneously with 150 c.c. serum 1715; and on the third day 225 c.c. serum 1715. Again a slight reaction took place, lasting from the third to the tenth day. The horse was discharged on the 30th October, 1917, from the experiment and sent to Doornpoort farm.

Result.—The infusion of blood of a horse that suffered from acute Liver-atrophy did not produce this disease in the horses injected. One of the horses, however, contracted Horse-sickness. The blood of a horse immunized against Horse-sickness thus proved to be virulent 66 days after injection of virus. The remarkable observation was made here that the second horse (10955), being a horse susceptible to Horse-sickness, as the subsequent history proved to be, did not develop the disease from the infusion of the blood of horse 10209, which proved virulent for the first horse.

II. *Experiment*.—Infusion of Staggers blood into horses 11013 and 11030.

Horse 10884.—(For history of this horse *vide* Appendix I.)—This horse developed acute Staggers on the 14th March, 1917, on which date its blood was transfused into two susceptible horses, 11013 and 11030, the former receiving a total amount of 4 litres (8 minutes) and the latter 3 litres (6 minutes).

(1) Horse 11013, a three-year-old brown gelding, in fair condition, arrived in Onderstepoort on the 21st February, 1917, and was kept under observation until the date of infusion on 14th March, 1917. The temperature record at the beginning of this period did not run a normal course; slight disturbances were present. The latter period was, however, normal. Seven days after infusion the temperature rose to 104° F., and remained at that average to

the tenth day (24th March), when the horse died. On the 22nd March crusts of dried blood were noticed on the left nostril, and on examination with the speculum it was noticed that the blood originated in the upper nasal region. In the morning of the 24th March this haemorrhage was still present. The respirations numbered 30 per minute and the auscultation revealed increased vesicular murmur. The horse died during the night of Horse-sickness. This diagnosis was supported by a subsequent test of its blood into a susceptible horse 11015, that received 25 c.c. intrajugularly and died on the eighth day of Horse-sickness. In this particular case the blood of horse 10884 produced Horse-sickness 71 days after the first and 65 days after the second injection of virus "T." or "O." respectively.

(2) Horse 11030, an aged grey gelding in fair condition, arrived in Onderstepoort on the 6th March, 1917, and was kept on temperature record from that date. On the 14th March, 1917, it was infused. The registering of the daily temperature was continued, but did not show any abnormalities, and the horse was in perfect health. On the 12th July, 1917, the horse was immunized for Horse-sickness, receiving an intrajugular injection of 5 c.c. virus of horse 11323, Tzaneen, and on the 7th and 9th day serum 1715 respectively 160 and 225 c.c., and a second injection of virus of three different origins. The horse had a slight reaction from the 5th to the 13th day, which must be interpreted as a Horse-sickness reaction, although no further symptoms were present. On the 7th August, 1917, the horse was discharged from experiment.

Result.—Of the two horses infused with blood from a horse suffering from acute Staggers none developed this disease. One, however, contracted Horse-sickness and died, whilst the second remained healthy. Also here the remarkable observation was made that one horse infused (11013) contracted Horse-sickness whilst the second horse did not, although succeeding events proved it to be susceptible for Horse-sickness. In this instance the blood of an immunized horse contained the virus of Horse-sickness 71 days after the injections of the virus.

III. Experiment.—Injection of Liver Emulsion of Staggers horses into horses 10891, 11011, 11024, and 10690.

(1) Horse 10891, an aged cream stallion of good condition, had been immunized against Horse-sickness on the 20th December, 1916, and showed the symptoms of Dikkop. Its temperature after this was constantly normal. On the 14th March, 1917, it was injected into the jugular vein, receiving 100 c.c. emulsion of the liver of horse 10884; the emulsion was made by passing the liver tissue through a Latapie apparatus and mixing it with physiological water. There were only slight temperature disturbances subsequent to this and of no definite character, and the horse kept in perfect health. It was subsequently used in a *Crotalaria dura* feeding experiment and died of Jagziekte on the 2nd September, 1917.

Result.—The injection of an emulsion of liver tissue of a horse that died of acute Liver-atrophy did not produce this disease in the horse injected.

(2) Horse 11011, a *not immunized* ten-year-old chestnut gelding in fair condition, arrived in Onderstepoort on the 21st February, 1917, and was kept under observation until the 14th March, 1917, when it received, in the jugular vein, 150 c.c. of liver emulsion of horse 10844. The injection was well supported. Subsequently the same horse was used for the second experiment with liver emulsion of horse 10831 (*vide infra*). No symptoms of any kind were noted up to the date of the second experiment, and only a little after that (*vide infra*). On the 12th July, 1917, the horse was immunized against Horse-sickness, when it reacted to the second virus with a fever curve that must be considered typical of Horse-sickness. On the 7th October, 1917, the horse was discharged from experiment.

Result.—The injection of an emulsion of liver tissue from a case of acute Liver-atrophy into a horse did not cause this disease. This horse was susceptible to Horse-sickness. The liver emulsion did not cause Horse-sickness, which might have been expected in the light of the result from the blood infusion in horse 11013.

(3) Horse 11024, an aged bay gelding, fair condition, not immunized against Horse-sickness, arrived in Onderstepoort on the 21st February, 1917, and was kept on daily temperature record until the 14th March, 1917. During the first part of this period the temperature was not regular, but no cause could be

found to explain it. The horse received in the jugular vein on the 14th March, 1917, an injection of an emulsion of 500 c.c. liver tissue of horse 10884. The horse showed some uneasiness due to this injection in the way of laboured abdominal respiration and accelerated pulse. These symptoms, however, soon passed off. No temperature disturbance was noted after this, and the horse, having been under daily observation until the 12th July, 1917, was then immunized against Horse-sickness. It reacted with a typical Horse-sickness curve, and thus proved to be a susceptible horse. It was discharged on the 7th October, 1917.

Result.—A large quantity of liver tissue emulsion was injected into this horse without transmitting Liver-atrophy. Here it is also interesting to note that the liver emulsion did not contain Horse-sickness virus.

(4) Horse 10690, an aged bay stallion in very good condition, arrived in Onderstepoort on the 29th September, 1916. It was immunized against Horse-sickness. On the 14th March, 1917, it was injected in the jugular vein, receiving 50 c.c. liver emulsion of horse 10884. The temperature after this kept normal, and the horse was in perfect health up to the 14th June, 1917, when it was placed in a *Crotalaria dura* feeding experiment. In October, 1917, the horse was still alive.

Result.—The injection of a liver tissue emulsion did not transmit the disease acute Liver-atrophy to a horse that was immunized against Horse-sickness.

IV.—*Experiment.*—Infusion of Staggers blood into horse 10918.

Horse 10831.—(For history of this horse *vide* Appendix I.)—This horse developed an acute attack of Staggers on the 24th March, 1917, and an infusion of blood was made into horse 10918.

(1) Horse 10918, an aged grey gelding, not immune, in moderate condition, at Onderstepoort since the 15th December, 1916, maintaining during this period a normal course of temperature. On the 24th March, 1917, blood of horse 10831 was infused into its jugular vein for 8 minutes (4 litres). The horse developed a fever reaction from the next day, resembling a curve with a short incubation period that might be interpreted as a Horse-sickness reaction. On the fifth day parasites of *Nuttallia equi* were found and were present during a number of days. The horse was treated with bihydrobromide of quinine and recovered. Since then it was kept under further observation, and on the 22nd June, 1917, it was tested with the Tzaneen virus of mule 10711, to which it did not react. It was tested with virus "O." on the 30th December, 1917, contracted Horse-sickness and died.

Result.—The infusion of Staggers blood of horse 10831 did not cause the appearance of that disease in the infused horse (10918). It cannot definitely be stated whether the reaction after the infusion was Horse-sickness. It is most likely that it was so, notwithstanding the presence of *Nuttallia* during the reaction, which may be accidental and notwithstanding the horse proving subsequently immune to the Tzaneen virus of mule 10711; Tzaneen immunity does not always protect horses against "O." virus.

V. *Experiment.*—Injection of Liver Tissue Emulsion of the Staggers horse 10831 into horses 10894 and 11011.

(1) Horse 10894, a not immunized aged brown gelding, purchased on the 12th December, 1916. It passed the mallein test on the 18th December, 1916, with negative results, and was kept on daily temperature record until the 6th February, 1917, during which time normal conditions were noted. On that date it was submitted to a pernicious Anaemia experiment, receiving intrajugularly 25 c.c. blood of horse 9406 (5th October, 1915), blood which proved to be virulent for horse 9433. There was no reaction up to the 45th day (i.e. the 24th March, 1917), when it was utilized for the injection of a liver tissue emulsion of horse 10831, dead of acute Liver-atrophy, receiving intrajugularly 600 c.c. emulsion of 250 gr. liver (passed through a Latapie apparatus) and 1000 c.c. physiological water. A remittent fever from the first to the eighth day was noted, maximum temperature 104.6° F. on the fourth and fifth days respectively, with a pulse of 52. A further reaction started suddenly on the

27th day and continued with irregular exacerbations to 105° F. until the 38th day. The symptoms of an acute lymphangitis were noted on the day after the onset of the fever. On the 23rd April, 1917, the off limb was markedly swollen from the region of forearm downwards. The skin was tense, the swelling hot, and the animal pointed the foot, going distinctly lame. Blood was drawn during the reaction and injected into horse 11337 (which died 35 days later from acute pernicious Anaemia). From the 30th April, 1917, onwards the swelling in the forearm of horse 10894 disappeared, the temperature dropped, the knee remained much swollen and painful, and a distinct falling off in condition was noted. For a number of days the anus became relaxed and no temperature could be recorded. During this period the animal became noticeably ill, the pulse reaching 80 beats per minute. The mucous membrane became pale yellowish tinged on the 8th May, 1917. The lameness did not disappear. The horse died on the same day, the clinical diagnosis of pernicious Anaemia having been made. The horse was tapped on the 32nd day after the infusion, viz., the fourth day of the second fever reaction.

Post-mortem.—Condition poor. The abdomen normal. Rigor mortis marked behind and slight in front. Integument: lesions of decubitus on temporal regions, shoulder, side of body, external angles of ileum. Pupils dilated. Mucous membranes pale, slightly yellow tinged. Couple of small abrasions on mucous membrane on inside of lower lip. Right forearm much swollen. Wound on outside of right carpus. Hind legs and fetlocks were slightly swollen. The flesh was dark red in colour and slightly brown tinged. The blood was dark red with brownish tinge, partially coagulated. Fat was practically absent, only a very small quantity was present. Connective tissue throughout the body showed an icteric tinge. The salivary glands and the lymphatic glands were normal. Tongue: petechiae, ecchymoses, and haemorrhages. Oesophagus and pharynx showed nothing unusual. Larynx and trachea showed slight congestion of vessels, the mucosa was yellowish. Fibrous filaments were present on both surfaces of diaphragm and also some ecchymoses and petechiae on peritoneal surface. Pleural cavities: petechiae and ecchymoses were sprinkled along dorsal parts of intercostal spaces and along the sides of the dorsal vertebrae. Respiratory organs: Left lung: not collapsed, costal surface showed fibrous filaments, ecchymoses and petechiae. On section stasis was noted. Bronchus mucosa was pale, yellow tinged. Right lung: the same as the left. The blood in the artery was coagulated. Some emphysema was present in anterior lobes of both lungs. Prepectoral glands were enlarged and oedematous, very slightly hyperaemic. The aorta showed nothing unusual. Pericardium contained 50 c.c. slightly turbid liquid, orange stained with a red tinge. Heart: both ventricles distended, the right ventricle more than left, filled with dark coagulated blood having the appearance of black currant jelly. Ostium was tight for the closed hand. Aorta and truncus brachiocephalicus were filled with jelly-like coagulum. Left ostium was tight for a fist. Coronary sulci were sprinkled with petechiae and ecchymoses. Right endocardium showed nothing unusual. Right wall was 1.4 cm. thick, slightly pale and opaque. Left endocardium showed ecchymoses on muscoli papillares and on septum. Myocardium was of a greyish brown red colour, left wall was 4 cm. thick. Consistence was fairly firm. Periportal glands were somewhat enlarged and haemorrhagic. Liver: fibrous filaments were present on anterior surface. The capsule was tense, retracting slightly on section. Consistence was very firm. Lobulation was fairly distinctly seen through the capsule. The centre of the lobules showed a marked greenish pigmentation. In some lobules the pigment was of an orange colour. Slight stasis was present in some of the central veins. Edges of liver were fairly rounded. Consistence of parenchyma friable. Pancreas showed nothing unusual. Splenic lymph glands were very much enlarged, oedematous, and hyperaemic. Spleen was very much enlarged, 59×30 cm. On section the pulp dark red, moist, jelly-like in consistency. Trabeculae not distinctly visible in places. Follicles were not enlarged. Suprarenal glands were enlarged, on section showed nothing unusual. Both kidneys were enlarged, the capsule was easily detached. The surface of the kidney appeared brownish, a fine reddish meshwork was noticeable due to congestion of vessels. On section the cortex was greyish yellow, the glomeruli appeared slightly red, projecting as pin-head points above surrounding surface. Medulla was slightly hyperaemic. Consistence was fairly firm. Stomach: on fundus patches of hyperaemia sprinkled with petechiae. Cardiac portion contained gastrophilus larvae. On duodenum and pylorus a thickening of the mucosa was present. Petechiae in the mucosa and haemorrhages in the submucosa were noted. Small intestines: mucosa showed a yellowish tinge, was slightly

thickened and wrinkled, especially in terminal portion, where there was also some hyperaemia. Large intestines: patches of deep red diffuse hyperaemia, rather extensive in places, mucosa otherwise greyish in colour. Ecchymoses and small extravasations were present in the mesentery and under the parietal peritoneum. Mesenteric lymph glands were very much enlarged, oedematous and hyperaemic. The anterior mesenteric artery contained a thrombus. Abdominal aorta showed nothing unusual. Urine was dark brown with a reddish tinge, and having the consistency of syrup. Sexual organs showed nothing unusual. Brain was normal. Molar teeth were regularly worn, first upper projecting very little beyond others in the row. Incisors regularly worn. The bone marrow of the humerus fatty, with red border. The femur: proximal spongiosa was red, shaft nine-tenths red.

Pathological anatomical diagnosis.—Fresh decubitus. Icterus. Tumour of the spleen; biliary pigmentation, fatty degeneration of liver, cloudy swelling and fatty degeneration of heart, very slight hydropericardium, petechiae, and ecchymoses on epicardium, petechiae, ecchymoses in left endocardium. Lung stasis. Fatty degeneration of kidney. Patchy hyperaemia and petechiae of the fundus mucosa. Petechiae and ecchymoses under parietal peritoneum and parietal pleurae. Haemoglobinuria. Hyperaemia in periportal, mesenteric and splenic lymph glands. Microscopical examination of spleen was negative for *Nuttallia equi*. A few Jollys bodies were found.

Etiological diagnosis.—Pernicious Anaemia.

Microscopical examination.—The section had a variegated appearance due to the presence of blood in the vessels and capillaries, the liver cells to a large extent being pigmented and many showing vacuoles. The peripheral cells of the lobules were pale, large round cell foci were present in the septa. The central veins were filled with blood, and their adjacent capillaries were distended in various degrees. In some places the distention of the capillaries was so pronounced that the liver cell rows were greatly reduced in size, forming narrow strips. In many places the distended capillaries dominated. The liver cells around the central veins and between the capillaries contained pigment so that the cells had an unusually dark appearance. They were apparently completely filled with the pigment and stood out prominently, and the atrophy of many of them was very distinct. The pigment was seen to be olive green in the Scharlach stained and light brown in the Haematoxylin-eosine stained section. Nearest the periphery the liver cells were free from pigment and they contrasted distinctly; moreover, they were light coloured and contained groups of nuclei. Some nuclei were surrounding a lumen, there were clusters without a lumen. Apparently the darker shaded liver cells originated from these young cells. The cells grouped around a lumen belonged to newly formed bile-ducts, which in some places were increased in numbers. In the septum was a rich collection of round cells extending alongside the periphery of the lobules and between the liver cell rows of the periphery. Amongst them were polynuclears; the majority were, however, round cells, some of large size. The Scharlach-stained section showed in some places the presence of fat globules in cells surrounding the central veins, in others they were placed in the intermediate portion of the lobule outside the blood pools, sometimes also in the periphery and some of the budding bile-ducts also contained fat granules. Kidney, right: the lumen of the tubuli contorti was filled with a homogeneous pink substance, a similar substance was present in the capsular space of the glomeruli, whilst the tufts were filled with red corpuscles. Some of the glomeruli were atrophied and some had a thickened capsule. In the adventitia of some of the vessels were small round cell collections. A considerable amount of blood was present in the intermediate zone. Scharlach showed only traces of fat in the epithelial cells. Left: here were some patches in which the tubules were replaced by connective tissue filled with round cells; these patches were running radially, including some glomeruli with thickened capsules. Heart: no fat was present. Small clusters of brown pigment were in the neighbourhood of the nuclei. Muscle: only occasionally a fibre showed a slight fatty degeneration. Two sarcosporidia were present. Spleen: much blood was present in the sinuses and some pigment. Suprarenal glands: a moderate amount of fat in the cortex and most in the zona glomerulosa and a fair amount of blood, so that in the Scharlach-stained section green and red streaks were seen alongside. Lymph glands: all the sinuses were filled with red corpuscles and serum. In the lumen of the vessels were plugs containing many leucocytes. Bone marrow: myeloblasts were very conspicuous. There was also some brown pigment present.

Diagnosis.—Fatty degeneration and pigmentation of the liver. Atrophy of liver cells, stasis; round cell infiltration. Nephritis interstitialis and fatty degeneration. Fatty degeneration of skeletal muscle. Brown atrophy of the heart. Hyperaemia and pigmentation of the spleen. Fatty degeneration of suprarenal glands. Oedema and haemorrhage of lymph glands. Pigmentation of bone marrow.

Etiological Diagnosis.—Pernicious Anaemia and acute Hepatitis parenchymatosa.

Result.—Subsequently to the infusion of an extract of liver tissue from a horse that had died of Staggers the infused horse died. The clinical symptoms were not those of acute Liver-atrophy but of pernicious Anaemia. The pathological picture found in the liver was diagnosed as parenchymatous Hepatitis, lesions akin to those of acute Liver-atrophy.

Subinoculations with blood from horse 10894 (*Experiment VA*).

(1) Horse 11337, a dark grey six-year-old gelding, in fair condition, received on the 20th June, 1917, an injection of 20 c.c. blood intrajugularly, taken on the thirty-second day after injection of horse 10894, viz., on the fourth day of the second fever reaction. The temperature record was normal until the twenty-eighth day after this injection, when a fever curve developed, with a maximum exacerbation to 105.2° F. on the second day of the reaction. The symptoms of an acute pernicious Anaemia developed, and the horse was killed on the thirty-fifth day or the fifth day of the fever. Post-mortem examination supported the diagnosis.

Result.—Blood of the horse that subsequently died with lesions resembling Liver-atrophy—collected previous to death and during a fever reaction—produced pernicious Anaemia in the horse injected.

(2) Horse 11011.—(For previous history *vide Experiment III supra*.)—On the 25th March, 1917, this horse received an injection into the jugular vein of 300 c.c. liver tissue emulsion, made of 250 grm. of liver of horse 10831 (acute Liver-atrophy) in 1000 c.c. physiological water. On the fourth and fifth day after the injection a disturbance in the temperature was noted. Thereafter normal conditions prevailed, when on the 12th July, 1917, it was immunized against Horse-sickness and reacted to the injection.

Result.—The injection of an emulsion of liver tissue did not transmit Liver-atrophy, neither did it produce Horse-sickness.

VI.—*Experiment.*—Infusion of Staggers blood into horses 11057 and 11055.

Horse 10854.—(For complete history of this horse *vide Appendix I*.)—This horse developed an attack of acute Staggers on the 3rd April, 1917, and on the 4th four litres of its blood were infused into horse 11057 and horse 11055. Horse 10854 was then killed, and an emulsion was made from the liver tissue and infused into horse 11054.

(1) Horse 11057, an aged grey gelding, purchased on the 29th March, 1917. After submitting to the mallein test on the 30th March, 1917, with negative result, it was kept on daily temperature record and normal conditions were noted. On the 4th April, 1917, it received an infusion of 4 litres of blood from horse 10854. A reaction developed from the fifteenth day onwards. The fever was remittent and lasted from the fifteenth to the twenty-sixth day. Maximum temperature was 104.4° F. on the twenty-second day. *Nuttallia equi* was noted in rare numbers on the twenty-first day. The lesions of anisocytosis and slight poikilocytosis were also noted. Another reaction occurred from the thirty-eighth day. Maximum temperature 103° F. on the fortieth and forty-first day; the pulse during this reaction reached 60 per minute. The cardiac impulse was increased and extended. Hind limbs were swollen. The mucous membranes were noted pale since the onset of the first fever and remained so. Later they were yellowish and finally turned orange and became echymosed. On the forty-sixth day the horse went down and was unable to rise and was destroyed. The clinical diagnosis of pernicious Anaemia was made.

Post-mortem.—Condition was poor. Rigor mortis was absent. Abdomen showed nothing unusual. Slight fresh decubitus was present on hips. Natural openings and the visible mucous membranes of lips and gums were pale yellowish. Subcutaneous tissue had a yellow tinge; blood was not coagulated.

Mandibular and retropharyngeal lymph glands and the thyroids showed nothing unusual. Bronchial and mediastinal lymph glands were slightly pigmented. Tongue tissue was yellowish; oesophagus was faintly yellow, also the pharynx mucosa. Peritoneal cavity: the serosa was yellowish, no fluid was present. Diaphragm convexly forwards, smooth, and slightly yellowish. Pleural cavity contained no fluid. Cervical trachea was yellowish, also the larynx. The right lung was half collapsed, pleura was yellowish and showed fibrous patches; on section nothing unusual was seen. Left lung was similar; one calcareous nodule the size of a pea was found. Intima of pulmonary vessels, thoracic trachea, and aorta were markedly yellow. Slight emphysema was present in right anterior lobe. Pericardium sac contained 150 c.c. clear yellow liquid. Heart: fluid blood was present in both ventricles. Epicardium had a yellow tinge, it showed petechiae at the base. Left endocardium was yellowish and showed marked ecchymoses. Right endocardium was also yellowish, valves were normal. Myocardium was pale yellowish. Periportal glands were yellowish. Liver: left lobe was markedly swollen, edges were rounded, fibrous filaments were present on capsule, also a few small white nodules in the parenchyma (parasitic), which was firm on section. It had a mottled appearance, the lobules were distinct; they showed a dark centre and a pale yellow periphery. The pancreas was pale yellowish. Splenic lymph glands were yellowish tinged. Spleen measured 50×26 cm. Pulp was dark in colour and soft, the trabeculae were fairly distinct, the capsule was yellowish. Suprarenal glands: cortex yellowish. Kidneys on section yellowish throughout, consistence friable, capsule rather adherent. The stomach was contracted; spiroptera were fairly frequent; a small spiroptera tumour and a few gastrophilus larvae were present. Small intestines: duodenum showed nothing unusual; jejunum and ileum were slightly hyperaemic in parts and yellow stained, a mucous deposit was present. Large intestines: caecum showed a light patchy hyperaemia, sclerostomes were fairly frequent, the colon was the same, the rectum was yellowish. Mesenteric lymph glands showed nothing unusual. The mesentery was yellowish. Branches of anterior mesenteric artery contained a thrombus and nematode larvae. The abdominal aorta was yellowish. Bladder: urine was brownish yellow and had the consistence of thin syrup; the mucosa was pale yellowish. Brain showed nothing unusual. Bone marrow of humerus was fatty and showed a haemorrhagic focus. The proximal end of the femur was haemorrhagic.

Pathological anatomical diagnosis.—General icterus and anaemia. Petechiae on epicardium; ecchymoses on left endocardium, hydropericardium. Tumour splenis. Fatty degeneration and pigmentation of liver. Slight enteritis.

Etiological diagnosis.—Pernicious Anaemia.

Microscopical examination.—Liver: the normal lobular picture had disappeared. The septa were thickened and lined by numerous budding bile-ducts. The central portion of the lobule was occupied by blood, mingled with detritus. It could be seen in the Scharlach-stained section that a great amount of light brown greenish pigment was present mingled with fat globules, but no definite structure could be made out. The budding bile-ducts occupied the greater portion of the lobule, penetrating from the periphery towards the centre. In some parts nearest the periphery a distinct lumen could be recognized, surrounded by newly formed cells; not so in the periphery, where there were large multinuclear cells. It is evident that these cells were meant to replace the broken-down liver lobules. In places it could be seen that the new cells followed the path between the existing capillaries, lining them on both sides; in some places old and new cells were mingled; they could easily be differentiated, the old ones by their darker colour. It could also be seen that the new cells caused the old ones to atrophy; in places between the new cells pigment was also present, not so much in the periphery as towards the centre. It seemed to give way to the advancing new liver cells. In the Scharlach-stained section it could be seen that many newly formed liver cells and bile-ducts showed infiltration with fat globules, the shape of the cells was, however, complete. In some lobules the greater part had been replaced by these newly formed cells, which were arranged in an almost regular radiating order. The septum was thickened by connective tissue fibres and round cell infiltration, also pigment could be found there: around the larger bile-ducts was a concentric arrangement of the connective tissue fibres. The central veins were somewhat thickened and the wall was hyaline. Right kidney: a number of glomeruli showed thickened capsules and atrophy of the tufts. There was a considerable vacuolation present in the epithelial lining of the

tubuli contorti, and in some places round cell collections in the adventitia of the vessels. Scharlach stain showed a considerable amount of fat in the tubuli contorti and to a smaller degree in the tubuli recti, also the cells of Henle's loops of the intermediary zone showed fat. Heart: the fibres were uniformly dusted with fat globules (Scharlach), so that the section had a reddish appearance. Suprarenal glands: there was a diffuse red staining of the cortex with Scharlach, which was patchy in appearance, due to the more intense staining of some portions where the fat globules were of a large size. Also the zona glomerulosa showed much fat. Muscle: the greater portion of the fibres showed a dusty appearance in Scharlach-stained section, so that macroscopically the red staining was discernible. Pancreas: in some portions of the glands a slight fatty degeneration was present. Spleen: much blood was in the sinuses and there were grains of pigment present.

Diagnosis.—Fatty degeneration and necrosis of liver cells; pigmentation; emigration of round cells. Regeneration of bile-ducts and connective tissue. Fatty degeneration of the heart. Fatty degeneration of the suprarenal glands. Fatty degeneration of the skeletal muscles. Fatty degeneration of pancreas. Hyperaemia and pigment in spleen.

Result.—In this case the horse went down forty-six days after the infusion and was then killed. It showed on post-mortem extensive liver lesions, the regenerative bile-ducts being very conspicuous. The picture resembled that of acute Liver-atrophy, an unusually abundant development of bile-ducts being noted. Also lesions in other parenchymatous organs were present that are associated with acute Liver-atrophy. The case must be interpreted at least as one of parenchymatous Hepatitis. It does not follow of necessity that the lesions were produced by the infusion. It may be a coincidence pure and simple, viz.: this horse might have gone down without any previous infusion. The case was one of pernicious Anaemia, the tumor splenis in this respect being pathognomonic. The pernicious Anaemia lesions in the liver were overshadowed by the destructive processes present.

Subinoculation with blood from horse 11057 into horse 10965

[*Experiment VI (1) (a)*].

At post-mortem of horse 11057 on the 20th May, 1917, blood was collected and 20 c.c. were injected into the jugular vein of horse (a) 10965, an aged dark brown gelding that had been in Onderstepoort since the 27th December, 1916, and during which period it had shown a normal temperature record. No reaction resulted from this injection, and in August, 1917, it was immunized against Horse-sickness, undergoing a Horse-sickness reaction.

Result.—Blood collected at post-mortem of horse 11057 and injected into a susceptible horse did not transmit Liver-atrophy. It neither transmitted pernicious Anaemia.

Subinoculation into horse 11342 [*Experiment VI (1) (b)*].

Whilst horse 11057 showed reaction it was tapped on the 25th April, 1917, and 5 c.c. of this blood was injected into the jugular vein of horse 11342 (an aged brown gelding). On the sixty-first day after the injection, viz., the 20th July, 1917, a fever reaction started of a remittent character, with an exacerbation to 105° F. on the sixty-third day. The mucous membranes during this period were orange tinged, and the pulse was recorded as 64 a minute. On the sixty-eighth day normal conditions were again present. The horse was subsequently immunized for Horse-sickness, to which it did not definitely react. It was still alive at the end of October, 1917.

Result.—It is most likely that horse 11342 had a slight attack of pernicious Anaemia, from which it recovered, and that this attack was brought on by the injection of the blood of horse 11057.

Subinoculation with blood of horse 11342 [*Experiment VI (1) (c)*].

Infusion of blood of horse 11342 into horse 11365.

On the 20th July, 1917, viz., sixty-six days after the injection of blood of horse 11057, viz., during a fever reaction, a transfusion of 5000 c.c. blood was made into horse 11365 (a brown mare in Onderstepoort since the 27th February, 1917). A reaction started immediately and lasted about eight days, the blood during this reaction showing *Nuttallia equi* in rare numbers. It was treated with bihydro-quinine. Subsequently a new fever reaction was noted of a short

duration. The horse was immunized for Horse-sickness on the 11th September, 1917, and reacted to the injection. The horse was still alive at the end of October, 1917.

Result.—Horse 11365 was infused in order to determine a reaction noted in horse 11342 subsequent to the injection of blood of horse 11057. A biliary fever reaction resulted. The horse was not immune to Horse-sickness. No symptoms of pernicious Anaemia developed. It does not follow that the reaction in 11342 was not pernicious anaemia, the infused horse 11365 was most likely immune against the disease.

Experiment VI (2).—Horse 11055, an aged grey gelding in good condition, was in Onderstepoort since the 30th March, 1917, with normal temperature records. On the 4th April, 1917, it was infused intrajugularly with 1650 c.c. defibrinated blood of horse 10854, then suffering from an acute attack of Staggers. From the sixteenth day onwards a fever reaction developed, which was typical for Horse-sickness; symptoms of Dikkop developed on the twenty-third day and were present on four days. A normal temperature then continued until the fifty-seventh day (the 31st May, 1917), when remittent fever developed, with high evening exacerbations and deep morning remissions. The horse became visibly ill, was feeding badly, and the mucous membranes had a washed-out appearance, the intermandibular lymph glands were tumified, and a discharge from the nostrils was present. The diagnosis of glanders was made and the horse was killed on the 19th May, 1917.

Result.—The infusion of blood of horse 10854 produced Dikkop in the infused horse. This horse subsequently developed acute glanders, which, however, must have been spontaneously contracted.

Subinoculation of blood 11055 into 11086 [*Experiment VI (2) (a)*].

During the Horse-sickness reaction, twenty-one days after injection of the defibrinated blood from horse 10854 (Staggers), horse 11055 was bled (25th April, 1917), and 5 c.c. were injected into horse 11086 (a brown gelding in moderate condition), which horse had been under observation since the 5th April, 1917. The injection was made on the 27th April into the jugular vein. A typical Horse-sickness reaction developed with the symptoms of Dikkop and the horse recovered. The horse was then kept under further observation until the 29th September, 1917, when it was killed, having become badly affected with mange.

Result.—The subsequent subinoculation of blood of horse 11055 again produced Horse-sickness, from which the animal recovered.

VII. Experiment.—Injection of Liver Tissue Emulsion of a Staggers Horse into Horse 11054.

(1) Horse 11054, a chestnut gelding in fair condition, received on the 4th April, 1917, intrajugularly an injection of 500 c.c. liver tissue emulsion of horse 10854 (made with 260 gm. of liver substance in 1200 c.c. water). The horse showed some disturbance immediately after the injection, which soon passed over. Ten days later, on the 14th April, 1917, a fever reaction started, which had a somewhat irregular course, showing exacerbations and remissions in no definite order. During the course of the disease the legs were swollen and the mucous membranes were pale yellowish, and a discharge from the nostrils was noted. The examination of the blood gave negative results. By about the twenty-eighth April the temperature curve showed again a normal level. A new reaction was noted to start on the 3rd May, 1917, and to last to the 15th May, 1917, during which period a greenish and purulent discharge of the nose was noted, the swelling of the hind limbs disappeared. A further slight reaction was noted on the 18th June, 1917, lasting a few days, when again the nasal discharge was present. On the 12th July, 1917, the horse was utilized for a Horse-sickness experiment, and it died of Horse-sickness on the 21st July, 1917.

Result.—The injection of an emulsion of liver tissue did not transmit the disease Staggers. Unusual symptoms developed that somehow could not be accounted for. It is possible that they were caused by the infusion of the liver emulsion. On post-mortem of the horse subsequently no explanation could be found for the symptoms, the lesions having apparently healed out by that time.

Subinoculation of blood of horse 11054 into horse 11049 [*Experiment VII (1) (a)*].

Horse 11054, which developed a reaction after the infusion of liver emulsion, was tapped on the 19th April, 1917, and on the 20th 20 c.c. blood were injected into the jugular vein of horse 11049, a seven-year-old grey gelding that had been under daily observation since the 30th March, 1917, during which period normal conditions were present. No reaction was noted after the injection. On the 11th August, 1917, the horse was immunized against Horse-sickness, responding with a typical Horse-sickness reaction, which was succeeded by the symptoms of Dikkop. The horse was discharged on the 11th September, 1917.

Result.—No results were obtained from the injection of blood of horse 11054 that developed unusual symptoms after the injection of liver emulsion.

VIII. *Experiment.*—Infusion of Staggers Blood into the horses 11327 and 11340.

Horse 10943.—(For complete history *vide* Appendix I.)—This horse developed an acute attack of Staggers on the 25th May, 1917, when its blood was transfused into the horse 11327 for ten minutes (5 litres), and in horse 11340 for four minutes (2 litres).

VIII. (1).—Horse 11327, an aged roan gelding in fair condition, was on daily temperature record since the 8th May, 1917, during which period it showed normal conditions. On the 25th May, 1917, it was infused for ten minutes (5 litres). From the sixth to the ninth day a sharp temperature curve, reaching 105° F. on the seventh day, was noted, and a second one started on the thirteenth day, which turned out to be one of Horse-sickness, the horse dying of that disease on the nineteenth day.

VIII (2).—Horse 11340, an aged dark brown gelding in fair condition, on daily temperature record since the 8th May, 1917, during which period it showed normal conditions, was infused on the 25th May, 1917, for four minutes (2 litres). On the eleventh day a fever reaction started, that turned out to be one of Horse-sickness, of which disease the horse died on the fifteenth day.

Result.—The interesting fact was observed that both horses contracted Horse-sickness from the infused blood, and both horses died. The infusion in one case amounted to 5 and in the other to 2 litres of blood.

IX. *Experiment.*—Infusion of Staggers Blood into horses 11326 and 11324.

Horse 11046.—(For complete history of this horse *vide* Appendix I.)—This horse developed an acute attack of Staggers on the 16th June, 1917. On the 18th June, 1917, its blood was transfused into horse 11326 and horse 11324, each for four minutes (2 litres), and blood for the collection of serum was taken at the same time.

IX (1).—Horse 11326, an aged brown mare in fair condition, was in Onderstepoort on daily temperature record since the 8th May, 1917, during which period normal conditions were present. Infusion for four minutes (2 litres) from horse 11046 took place on the 18th June, 1917. The daily temperature record was normal until the 27th August, 1917, when the horse was passed in a Horse-sickness experiment, first with a Tzaneen virus, to which it reacted typically with Horse-sickness, and on the 18th September, 1917, into a spontaneous Horse-sickness virus experiment, when the horse again reacted, this time with a Dikkop, from which it recovered. It was discharged from experiment on the 11th October, 1917.

IX (2).—Horse 11324, an aged chestnut gelding, arrived in Onderstepoort on the 7th May, 1917, since which date it was on daily temperature record, which was constantly normal. On the 18th June, 1917, it received a transfusion of blood for four minutes (2 litres) from horse 11046. All records thereafter were normal. On the 3rd October, 1917, it was injected with a Tzaneen virus, to which it reacted promptly with a typical Horse-sickness reaction and recovered. The horse was still under observation at the end of October, 1917.

Result.—The infusion of blood into two *not-immune* horses did not transmit the disease Staggers. Horse 11046, from which the infusion was made, being a non-immune horse, its blood could not be expected to contain the Horse-sickness virus.

X. *Experiment*.—Infusion of Staggers blood of horse 11409 into susceptible horse 11486 and immune horse 10673.

Horse 11409, an aged bay gelding in fair condition, arrived in Onderstepoort on 25th July, 1917, and was immunized on the 28th July, receiving only Tzaneen virus of horse 11374, and subsequently two injections of serum on the sixth and eighth day. It passed through a mild Horse-sickness reaction, but subsequently showed on one occasion (17 to 20 days after virus) a somewhat suspicious temperature exacerbation. On the 18th September, 1917, it received serum "O" and virus O simultaneously, and two days later again serum "O" to give the horse an "O" immunity. No reaction ensued. Sixteen days later the horse was discharged from experiment and sent to the farm Doornpoort. On the 27th November it developed acute Staggers and was then brought back to the laboratory, and the two horses were infused, horse 11486 receiving 2 litres of blood and horse 10673 receiving 750 c.c. blood. The horse then died, and pieces of the liver were emulsified and 500 c.c. of the emulsion were infused into immune horse 10853 and a susceptible horse 11484.

X (1).—Horse 11486, a brown gelding in good condition, arrived in Onderstepoort on the 26th October, 1917, and was placed under observation. On the 28th November, 1917, it was infused with 2 litres blood of horse 11409. Already four days later a temperature reaction commenced that lasted until the eighteenth day, and from the day after a second reaction started that ended in death. The diagnosis of pernicious Anaemia was made and confirmed by post-mortem examination, the disease being of a very acute nature.

X (2).—Horse 10673, a nine-year-old brown gelding, a horse in rather poor condition, arrived in Onderstepoort on the 20th September, 1916. It was immunized against Horse-sickness, receiving on the 17th November, 1916, Tz. virus 13 gen., and on the 24th November, 1916, "O." virus 176 gen. and serum, and a second dose of serum on the 26th November, 1916. It had a typical reaction from the tenth to the twentieth day. From the 2nd to the 15th July, 1917, it was used in a feeding experiment with *Crotalaria dura* with negative result. On the 28th November, 1917, it was infused with 750 c.c. blood of horse 11409. Irregular intermittent fever reaction resulted a few days after the infusion, and the animal died on the 20th January, 1918, of pernicious Anaemia, the diagnosis having also been made during life.

XI. *Experiment*.—Infusion of Liver Emulsion of horse 11409 into horses 10853 and 11484.

XI (1).—Horse 10853, an aged bay gelding, in rather poor condition, arrived in Onderstepoort on the 15th December, 1916. It was placed in a feeding experiment with *Crotalaria dura* with negative results. On the 28th November, 1917, it was infused with an emulsion made of 250 gr. liver tissue and 250 c.c. physiological water. Five days later an intermittent fever started that developed into a pernicious Anaemia reaction, and on 30th December, 1917, the horse died of pernicious Anaemia.

XI (2).—Horse 11484, an aged bay gelding in fair condition. It arrived on the 25th October, 1917, and was then placed under observation. It showed at intervals some irregular temperatures. On the 28th November, 1917, it was infused with liver tissue emulsion made as above. A temperature reaction started three days later of the pernicious Anaemia type, and the horse showed other clinical symptoms as well. A chronic form of pernicious Anaemia ensued, and the horse was still alive at the end of February, 1918.

Result of Experiments X and XI.—The blood and the liver tissue emulsion infused in all four horses developed typical pernicious Anaemia. Horse 11409 was accordingly a virus reservoir of pernicious Anaemia. It is interesting that in this case no Horse-sickness was noted, the horses infused were living long enough for its appearance. Three horses did not live long enough to exclude Staggers as a possible sequel; it did not develop in the fourth case.

XII. *Experiment*.—Infusion of Staggers blood into horses 11487 and 11499.

Horse 11414, a bay gelding, in fair condition, arrived in Onderstepoort on the 25th July, 1917. It was injected on the 31st July, 1917, intrajugularly, with 50 c.c. virus of a spontaneous Horse-sickness virus of horse 11336. No reaction ensued. On the 11th September, 1917, an intrajugular injection of 5 c.c. virus 11381 Tzaneen 20 generation, was made, again without any reaction. On the 25th September the horse was submitted to

an immunization experiment, receiving intrajugularly virus Tzaneen, six days later virus "O" and serum "O," and again serum on the ninth day. Quite an irregular reaction resulted, that could not be identified with Horse-sickness. It recovered, however, and by the 5th November, 1917, was sent to the farm. On the 17th December, 1917, it was returned as suffering from acute Staggers, and it died the same day. Previously to its death horses 11487 and 11499 were infused, each receiving 2500 c.c. of blood.

XII (1).—Horse 11487, an aged dark brown gelding, was immune to Horse-sickness, having resisted an intrajugular injection of "O" virus on the 28th of October, 1917. On the 17th December, 1917, it was infused with 2500 c.c. blood of horse 11414. Nothing was ever noticed to be amiss with this horse, and it was still alive at the end of February, 1918.

XII (2).—Horse 11499, an aged brown gelding, in good condition, arrived in Onderstepoort on the 9th November, 1917. For about the first ten days it showed an irregular fever reaction. On the 17th December, 1917, it was infused with 2500 c.c. blood from horse 11414, a reaction ensued lasting for a few days, and subsequently another disturbance was noted, but finally normal temperatures prevailed. On the 22nd January, 1918, the horse suddenly developed acute Staggers, and died as a result of it.

Result.—Staggers developed in one of the infused horses thirty-six days after infusion. *This is the first genuine case of uncomplicated Staggers noted after infusion of blood from a Staggers horse.*

XIII. *Experiment.*—Infusion of Staggers blood 11543 into susceptible horse 11509 and immune horse 11674.

Horse 11543, an aged grey gelding, in fair condition, arrived in Onderstepoort on the 3rd January, 1918. It was infused on the 7th January, 1918, receiving intrajugularly 5 litres blood of horse 11278, which had been immunized on the 7th November, 1917, against Horse-sickness both with Tzaneen and "O" virus, and had passed through a Horse-sickness reaction. A slight fever exacerbation on the evening of the thirteenth day after infusion was noted, and thereafter a normal temperature continued. On the 10th of February, 1918, the horse developed an acute attack of Staggers, to which it succumbed the same day. Previously to death the two horses were infused, horse 11509 receiving 2500 c.c. and horse 11674 2000 c.c.

(1) Horse 11509, a six-year-old bay gelding in fair condition, arrived in Onderstepoort on the 4th December, 1917, and was placed under observation. On the 10th February it was infused with blood 11543, receiving 2500 c.c. No disturbances were noted after this.

(2) Horse 11674, an aged bay gelding, in good condition. This was a police horse, having been immunized against Horse-sickness in Pretoria in 1916. Later it developed a paralysis of the facial nerve, and was taken over from the police for observation on the 4th February, 1918. On the 10th February it received 2 litres blood 11543, intrajugularly. Nothing happened.

Result.—The blood of the Staggers horse 11543 did not produce Horse-sickness in the susceptible horse 11509, and up to the date of writing neither of the two horses infused developed Staggers.

XIV. *Experiment.*—Injection of serum of Staggers horse 11046 (18th June, 1917), into five fresh horses.

(1) Horse 11298, an aged brown gelding, injected on the 27th June, 1917, into the jugular vein with 325 c.c. serum. (2) Horse 11225, a seven-year-old bay mare, injected intrajugularly with 325 c.c. serum on the 9th July, 1917. (3) Horse 11294, an aged dun gelding, received on the 9th July, 1917, 325 c.c. serum intrajugularly. (4) Horse 11297, an aged dark brown mare, received on the 9th July, 1917, 325 c.c. serum intrajugularly. (5) Horse 11301, a seven-year-old piebald mare, received on the 9th July, 1917, 325 c.c. serum into the jugular vein. The horses were all alive at the end of October, 1917, and during the period of observation no symptoms of Staggers were registered.

Result.—The injection of serum of a horse, taken during an attack of acute Liver-atrophy, into five susceptible horses, in doses as utilized in the immunization of horses against Horse-sickness, did not produce Staggers.

XV. (1)—*Experiment*.—Injection of serum of five horses that have recovered from an attack of Staggers into horses that had not been injected before.

The horses 9581, 9880, 10601, 10641, and 10717, that had had typical attacks of Staggers (*vide* history of these horses in Appendix I), were bled for serum on the 23rd January, 1917. The serum was injected intrajugularly as follows: Horse 10921, on the 25th January, 1917, 180 c.c., and on the 27th January, 1917, 250 c.c. serum; and horse 10889, 190 c.c. on the 29th, and 290 c.c. on the 30th January, 1917, at the rate of 0.5 c.c. per kilo weight for the first and 0.75 c.c. per kilo for the second injection. These horses were kept under observation, and the daily temperatures recorded for over six months, during which time no symptoms of Staggers developed. On the 1st February, 1917, the non-immune horses 10770, 10772, 10773, 10906 were injected, each receiving 500 c.c. of the serum intrajugularly. The horses were kept under observation for at least four months, during which period none showed symptoms of Staggers. On the 12th May, 1917, the non-immune horses 11110, 11111, 11112, 11113, 11114, 11115, 11116, 11117, 11118, 11119, 11120, 11121, 11122, 11123, 11124, 11125, 11126, 11127, 11128, 11129, each received an intrajugular injection of 325 c.c. serum.

Result.—None of these horses, which were kept under observation for at least five months, showed subsequently any symptoms of Staggers.

XV. (2) *Experiment*, with serum of one recovered horse 10641. (For history *vide* Appendix I.)

The horse had an attack of Staggers on the 4th January, 1917, and recovered. It was hyperimmunized with ten litres virus of horse 10966, "O" virus 184th generation. It was bled on the 20th February, 1917, for serum.

(a) *First injection of serum* (10641).—The serum was injected on the 10th and 12th March, 1917, into horse 10998 in the quantities of 160 c.c. and 240 c.c. respectively, at the rate of 0.5 c.c. and 0.75 per kilo weight; horse 11001 respectively 200 c.c. and 300 c.c.; horse 11002 respectively 225 c.c. and 325 c.c.; horse 11003 respectively 200 c.c. and 300 c.c.; horse 11004 respectively 165 c.c. and 250 c.c.; horse 11018 respectively 200 and 300 c.c. Of this lot, two horses, 11004 and 11018, developed fever reaction.

Horse 11004, an aged grey gelding, seven days after injection, showed a sharp reaction, reaching 105° F. on the ninth day, the fever lasting for five days. During this period the conjunctiva was orange tinged, the microscopical examination of the blood was negative. Subsequently no further symptoms developed, and the horse was immunized against Horse-sickness.

Subinoculation of blood 11004.—Blood (5 c.c.) of this horse, taken during the reaction on the tenth day, was injected intrajugularly into horse 11056 on the 2nd April, 1917. This horse showed an immediate slight temperature reaction lasting seven days, the maximum averaging 102° F. No other symptoms were noted.

Horse 11018, an aged bay gelding, showed two fever reactions, a slight one, from the fourteenth to the seventeenth day, of a remittent character, and a maximum record of 103.8° F. on the sixteenth day, and a second sharp reaction from the twenty-first to the twenty-sixth day, the maximum temperature reaching 106° F. on the twenty-second day. The pulse during this period numbered 68 on the twenty-second and twenty-third days, and 52 on the twenty-fourth day, when it was small and soft. After this no further reactions occurred, and the horse was put into a Horse-sickness experiment on the 12th June, 1917, and died of Horse-sickness.

Subinoculation of blood 11018.—During the reaction of this horse on the twenty-second day blood was taken, and 5 c.c. were injected intrajugularly into horse 11056 on the 23rd April, 1917. This horse was kept under observation until June, and no further symptoms were noted.

(b) *Second injection of serum* (10641).—On the 12th May, 1917, twenty horses (numbers 11130 to 11149) were injected, each horse receiving intrajugularly 325 c.c. The horses were kept under observation until the end of October, 1917, and during this period no symptoms of disease were recorded against them. One horse died subsequently of Horse-sickness on the 26th September, 1917, as a result of an experiment.

(c) *Third injection of serum* (10641).—On the 27th June, 1917, five horses were injected with 325 c.c. serum into the jugular vein, viz., numbers 11284, 11291, 11292, 11296, and 11302. These horses were still under observation at the end of October, 1917, and during this period no symptoms were recorded against them.

Result.—None of the horses injected with serum obtained from horses recovered from acute Liver-atrophy developed this disease. In two cases fever reactions were noted that could not be definitely diagnosed. It is likely that these fevers were not directly connected with the injection and more than probably of an intercurrent nature.

XVI. Experiment.—Injection of serum "O." 1715, Horse-sickness serum made on the 9th May, 1916, and utilized in the immunization of horses against Horse-sickness, amongst which Staggers had been noted both at Onderstepoort and in the practice.

(a) On the 5th March, 1917, and 7th March, 1917, ten horses were injected into the jugular vein: Horse 11000 received 180 and 270 c.c. respectively; horse 11006 received 190 c.c. and 282 c.c. respectively; horse 11007 received 215 c.c. and 325 c.c. respectively; horse 11008 received 185 c.c. and 280 c.c. respectively; horse 11009 received 200 c.c. and 300 c.c. respectively; horse 11010 received 185 c.c. and 280 c.c. respectively; horse 11012 received 170 c.c. and 250 c.c. respectively; horse 11014 received 180 c.c. and 270 c.c. respectively; horse 11017 received 180 c.c. and 270 c.c. respectively; and horse 11020 received 205 and 305 c.c. Of these horses two reacted, viz., 11014 and 11020. Horse 11014, an eight-year-old dark brown gelding, showed, twelve days after injection, a sharp remittent fever, the evening exacerbation reaching a maximum of 105° F. and an average of 104° F.; the morning remissions were at an average of 100° F. The conjunctiva had an orange tint and was ecchymosed, a swelling was noted in the sternal region. Subsequently the temperature was of a remittent character, with evening exacerbation averaging 102° F. to 103° F., but no other symptoms were recorded. This horse died on the 21st July, 1917, from tympanitic colic.

Subinoculation of blood 11014.—During the fever reaction, the fifteenth day after injection, horse 11014 was bled and 5 c.c. of the blood were injected on the 20th April, 1917, into horse 11042 intrajugularly. No symptoms were recorded in this horse.

Horse 11020, an aged chestnut gelding, developed thirteen days after injection a sudden fever reaction, starting with 103.4° F. in the evening. The succeeding days the average temperatures were less. The fever passed over in four days. The conjunctiva was slightly congested during this period, and a pulse of 48 was registered on one occasion. The horse was kept under further observation, and in July, 1917, was used in a Horse-sickness experiment and died of Horse-sickness.

Subinoculation of blood 11020.—During the fever reaction on the fifteenth day the horse was bled. 5 c.c. of this blood were injected intrajugularly into horse 11042 on the 23rd April. No symptoms were registered against this horse, and it was still alive at the end of October, 1917.

(b) On the 10th April, 1917, and 12th April, 1917, six horses received an injection into the jugular vein, viz.: Horse 11044 received 185 c.c. and 275 c.c. respectively; horse 11046 received 220 and 325 c.c. respectively; horse 11051 received 195 c.c. and 295 c.c. respectively; horse 11060 received 190 c.c. and 285 c.c. respectively; horse 11064 received 205 c.c. and 310 c.c. respectively; and horse 11065 received 195 c.c. and 295 c.c. respectively. Of these horses, one, 11046, an aged bay gelding, developed Staggers on the 17th June, 1917, viz., 68 days after injection (history of this horse *vide* Appendix I). The rest of the horses showed no symptoms during the period they were under observation, and at the end of October, 1917, were used for other experiments.

(c) On the 12th May, 1917, twenty horses were injected, viz., the numbers 11150-11169, each receiving intrajugularly 325 c.c. serum "O." 1715. These horses were still under observation at the end of October, 1917, and during this period no symptoms were registered against them.

(d) On the 27th June, 1917, twenty horses were injected with serum 1715, each horse receiving 325 c.c. intrajugularly, viz., the horses 11171, 11176, 11177, 11185, 11187, 11188, 11190, 11196, 11197, 11203, 11204, 11207, 11212, 11224, 11227, 11228, 11232, 11233, 11236, and 11238. One of these horses, 11188, a six-year-old bay gelding, showed on the 20th day an exacerbation to 105° F. on the 24th, 105.6° F. on the 26th, 105.2° F., and the 29th 106° F. The remissions in between were normal, so that the curve resembled somewhat that of an intermittent fever. On one occasion the pulse was 60. No other symptoms were noted, and the microscopical examination gave negative results. In the subsequent period no symptoms were recorded against this horse. It was immunized against Horse-sickness at the beginning of October, 1917.

Result.—One of the horses injected with serum 1715, viz., 11046, developed acute Staggers and died. Its blood was utilized for transfusion experiments without any further results. This one case finds probably the right explanation by considering it as a mere coincidence. The greater the number of horses used for infusion, the greater the chances that amongst them were some that already had the disease in their system. In some horses intercurrent fever reactions were noted, that must not be connected with the serum injection.

XVII. Experiment.—Injection of serum "O" 1717, Horse-sickness serum made on the 10th July, 1916, and utilized in the immunization of horses against Horse-sickness amongst which no Staggers was noted at Onderstepoort, but amongst horses in the practice.

On the 27th June, 1917, twenty horses were injected intrajugularly with serum "O" 1717, each horse receiving 325 c.c., viz., the horses 11242, 11246, 11247, 11250, 11252, 11253, 11254, 11256, 11257, 11263, 11264, 11265, 11266, 11272, 11273, 11275, 11276, 11277, 11278, 11282. Of these horses, one, 11253, a bay mare, five years old, developed a fever reaction seven days after injection, the curve having an irregular course, at times remittent, with a maximum exacerbation to 105.3° F. on the 28th day. The pulse varied between 36 and 56 on different occasions. On the 15th day swelling of the off hind leg was noted below the hock, causing lameness. This swelling decreased in the course of the next few days. The conjunctiva on the 16th day was noted to be pale, it took on a washed-out appearance subsequently, and on the 30th day was distinctly orange. The horse lost condition and got weak, dragging the hind legs when walking. The temperature was normal by the 37th day. Blood examination at intervals gave negative results. The horse recovered, and no further symptoms were registered against it. It was immunized in October, 1917, against Horse-sickness.

Subinoculation of blood 11253.—On the 14th day after the injection, blood of horse 11253 was infused into 11386, a five-year-old bay gelding, for 10 minutes (5 litres). The horse was in very poor condition. It was killed on the ninth day after injection. There were no symptoms recorded against it during this period.

Result.—Serum 1717 did not produce any cases of Staggers. One horse developed an intercurrent disease that possibly is not directly connected with the injection of the serum, it may have been a relapse of pernicious Anaemia.

SUMMARY OF EXPERIMENTAL RESULTS.

The blood from nine horses, that were suffering at the time of acute Liver-atrophy, was infused into fourteen horses which were not immunized against Horse-sickness. Of the nine Staggers horses, eight had been immunized previously against Horse-sickness, the longest interval between the last injection of virus and death being 77 days. The ninth horse had only been injected with serum. The blood of the eight immune horses produced Horse-sickness in six horses, of which four died; one horse died 46 days subsequent to the infusion, and on post-mortem showed the lesions of a parenchymatous Hepatitis and pernicious Anaemia (11057). Subsequent inoculations with the blood of this horse gave negative results as far as Liver-atrophy, but positive ones as far as pernicious Anaemia is concerned. One horse died of Staggers (11499).

Portions of the liver of four horses that died of acute Liver-atrophy were put up in emulsions and injected into eight horses, of which three were immune horses. In one instance, forty-five days after the injection, a horse died showing lesions of pernicious Anaemia, but at the same time also the lesions of parenchymatous Hepatitis were fully developed (10894).

Serum of a horse that suffered from acute Liver-atrophy was injected into five horses without any disease being produced.

Serum of one horse that had recovered from acute Liver-atrophy was injected into thirty-one horses, and none of them developed the disease.

Serum of five horses that had recovered from acute Liver-atrophy was injected into 26 horses, and none of them contracted the disease.

Serum that had been utilized for the immunization of horses, amongst which acute Liver-atrophy was noted, was injected into 76 horses, and one of these contracted the disease.

SUMMARY OF EXPERIMENTAL RESULTS.

No. of Horse.	Description and Age.	Received.		Immune or not.	Infusion.		Result.	Death.	Days since infection.	Remarks.
		Date.	From.		Date.	Quantity.				
10954	Aged chestnut gelding.	21. 12. 16	Defence	Not immune	27. 2. 17	5000 c.c. blood of H. 10209	Died of Horse-sickness.	13. 3. 17	14	Reacted subsequently to Horse-sickness immunization.
10955	Aged bay gelding.	" "	"	"	" "	" "	No reaction.	—	—	
11013	3 years brown gelding.	21. 2. 17	Silberman	"	14. 3. 17	4000 c.c. blood of H. 10884	Died of Horse-sickness.	24. 3. 17	10	Reacted subsequently to Horse-sickness immunization.
11030	Aged grey gelding.	6. 3. 17	"	"	" "	3000 c.c. blood of H. 10884	No reaction.	—	—	
10891	Aged cream stallion.	15. 12. 16	"	Immune	" "	100 c.c. liver emulsion of H. 10884	"	—	—	
11011	10 years chestnut gelding	21. 2. 17	Goldstein	Not immune	" "	150 c.c. liver emulsion of H. 10884	"	—	—	Reacted subsequently to Horse-sickness immunization.
					4. 4. 17	300 c.c. liver emulsion of H. 10831	"	—	—	
11024	Aged bay gelding.	" "	Silberman	"	14. 3. 17	500 c.c. liver emulsion of H. 10884	"	—	—	Reacted subsequently to Horse-sickness immunization.
10690	Aged bay stallion.	29. 9. 16	"	Immune	" "	50 c.c. liver emulsion of H. 10884	"	—	—	
10918	Aged grey gelding.	15. 12. 16	"	Not immune	24. 3. 17	4000 c.c. blood of H. 10831	Irregular reaction, with Nuttall's is	—	—	Died later, Horse-sickness.
10894	Aged brown gelding.	" "	"	"	" "	500 c.c. liver emulsion of H. 10 31	Died of pernicious Anaemia.	8. 5. 17	45	On autopsy, pericnchymatous Hepatitis.
11057	Aged grey gelding.	29. 3. 17	"	"	4. 4. 17	4000 c.c. blood of H. 10854	Killed for pernicious Anaemia	20. 5. 17	46	Died later, Glanders.
11055	Aged grey gelding.	" "	"	"	" "	1650 c.c. blood of H. 10854	Diklo and recovered	—	—	Died later, Horse-sickness.
11054	Aged chestnut gelding.	" "	"	"	" "	500 c.c. liver emulsion of H. 10854	Irregular reaction and recovery	—	—	
11327	Aged roan gelding.	8. 5. 17	Goldstein	"	25. 5. 17	2000 c.c. blood of H. 10943	Died of Horse-sickness.	13. 6. 17	19	
11316	Aged dark brown gelding	" "	Silberman	"	" "	5000 c.c. blood of H. 10943	"	9. 6. 17	15	Reacted subsequently to Horse-sickness tests.
11326	15 years brown mare.	" "	Goldstein	"	18. 6. 17	2000 c.c. blood of H. 11046	No reaction.	—	—	
11324	15 years chestnut gelding	25. 10. 17	"	"	28. 11. 17	500 c.c. liver extract of H. 11409	Pernicious Anaemia and recovered	—	16	Still alive.
11481	Aged bay gelding.	" "	"	"	" "	2000 c.c. blood of H. 11409	Died of pernicious Anaemia.	17. 12. 17	19	
11486	Aged brown gelding.	26. 10. 17	Silberman	Immune	" "	750 c.c. blood of H. 11409	Pernicious Anaemia.	21. 1. 18	54	Killed.
10673	9 years brown gelding.	20. 9. 16	Defence	"	" "	500 c.c. liver emulsion of H. 11409	"	30. 12. 17	32	
10855	Aged bay gelding.	15. 12. 16	"	"	" "	2500 c.c. blood of H. 11414	Died of Stagers.	23. 1. 18	37	Still alive.
11490	Aged brown gelding.	9. 11. 17	Silberman	Not immune	17. 12. 17	2500 c.c. blood of H. 11414	No reaction.	—	—	"
11487	Aged dark brown gelding	26. 10. 17	"	Immune	" "	2500 c.c. blood of H. 11414	No reaction up to 6. 3. 18.	—	—	"
11509	6 years bay gelding.	4. 12. 17	"	Not immune	10. 2. 18	2500 c.c. blood of H. 11543	No reaction.	—	—	"
11674	Aged bay gelding.	4. 2. 18	Defence	Immune	" "	2000 c.c. blood of H. 11543	No reaction.	—	—	"

APPENDIX IV.

SENECIO POISONING IN CATTLE.

IN 1906 feeding experiments with *Senecio burchelli* were undertaken by Chase on two young oxen and a calf. One ox received daily, for four days, 4 oz. (1 lb.) of the fresh succulent plant just after the rains. On the fourth day the animal was noticed ill, showing uneasiness, diarrhoea and straining (eventually the rectum became everted), dying at daybreak of the following morning. On post-mortem the liver was found in a state of acute venous congestion. The gall bladder was distended and inflamed. Leaves of abomasum were very much thickened and oedematous, due to the presence of a clear straw-coloured fluid under the mucous membrane, and over the surface were numbers of ecchymoses, in several places approaching to ulceration. Rectum was much congested. The heart showed epi- and endocardial haemorrhages. The second ox received half a pound for four days (2 lb.); diarrhoea appeared the third day, and the fourth day the animal commenced to strain, continuing all day. Death supervened on the fifth day. Post-mortem showed inflammation of the abomasum, thickening of the leaves by submucous effusion. Large red areas of extravasations were present. The gall bladder was distended and its mucosa showed petechiae. The liver showed lesions of acute venous congestion. The calf received small quantities of *Senecio burchelli* extending over a period of two months; this animal became so emaciated that it had to be slaughtered, being unable to stand. On post-mortem the liver had a sunken appearance, it was slate coloured and tough to the touch, firm on section; the gall bladder contained 6 oz. of dirty orange-coloured bile, thick and sticky, it could be pulled out like birdlime. The abomasum showed a few petechiae, no oedema. Other organs were normal.

The species *Senecio latifolius* was likewise submitted to tests in Capetown by Robertson, and the following results were obtained: A young ox received daily 2 oz. of the plant for thirty days when the quantity was increased to 4 oz. daily. Thirteen days later the ox started purging and straining, the faeces had a most offensive odour; the purging continued for fourteen days, when the ox became unable to rise, straining violently, grinding the teeth and twitching the eyelids, dying in the evening of the same day. Post-mortem showed the liver smaller than normal, hard, firm, and cirrhotic; on section the cut surface presented a pale appearance with areas of congestion. The gall bladder was much distended and full of bile (2 pints), thick and viscid in consistency, like birdlime. Abomasum showed the lining much congested and thickened, the mucous folds being distended with a clear coloured fluid and having a number of areas of inflammation. The intestines were pale and quite empty. Rectum was slightly everted and congested.

The second ox received daily 3 oz. of the plant for thirty days when the quantity was increased to 6 oz. Eleven days later the animal started purging, strained most violently (everting lower part of rectum) and fell down, it seemed unable to rise and unable to stand when lifted, it paddled with the feet, the eyes were twitching, the teeth grinding, and there was frothing at the mouth. It died the next day. Post-mortem showed a hard and cirrhotic liver, cutting with a firm sound. Gall bladder distended and full (3 pints) of pale, yellow, thick, sticky bile. Abomasum contained clear straw-coloured fluid.

FEEDING EXPERIMENTS WITH *Senecio latifolius* AT ONDERSTEEPOORT.

Experiment No. 1.—An eighteen-month old heifer (3518) which had been on the station since the 1st April, 1915, was given 8 oz. of Ragwort from the 21st February, 1916. She fed well for two days, and was found dead in the morning of the 23rd February.

Post-mortem.—Condition fair. Rigor mortis present. Visible mucous membranes were normal. Flesh slightly pale. Subcutaneous tissue: the blood-vessels were slightly injected and some extravasations were present. Diaphragm showed extensive adhesions to the reticulum and to the right lung. Peritoneal cavity: the omentum showed extravasations. Lymph glands: axillary and prescapular were oedematous and haemorrhagic, submaxillaries enlarged, oedematous, and hyperaemic; surrounding connective tissue was haemorrhagic, retropharyngeals were also hyperaemic. Thyroids normal. Tongue normal. Oesophagus normal. Some mucus was present in the pharynx. Respiratory organs: lungs were not collapsed, posterior lobe showed extensive adhesion to diaphragm and gelatinous infiltration, on section slightly oedematous. Pleura

was injected and numerous ecchymoses were present; on section lung showed slight oedema and some haemorrhages were present. Mucosa of thoracic trachea showed petechiae. Pericardium contained 125 c.c. dark red liquid. Circulatory organs: both ventricles were empty. Epicardium was covered with extravasations. Myocardium slight brownish, consistence was normal. Thoracic aorta was normal. Liver: adhesions to diaphragm; white foci on edges of liver on section, lobules fairly distinct with symptoms of stasis; colour of parenchyma was somewhat brown. Bile was green and liquid. On the dorsal edge of liver was an abscess with caseous pus. Spleen measured 40×11 cm., on section pulp slightly softened; trabeculae were fairly distinct. Kidneys: capsule was easily detached, on section normal. Stomach: walls of abomasum mucosa were showing numerous petechiae and ecchymoses. Submucosa was oedematous and haemorrhagic, contents were dry. In reticulum two pieces of wire were found; one piece was penetrating the wall bordering the diaphragm. In the small intestines were blood-stained contents, the mucosa was thickened and haemorrhagic. Large intestines: fibrinous coagula and petechiae in the mucosa. Rectum showed blood-stained contents. The bladder was distended with normal urine, mucosa showed some petechiae. The sexual organs were normal. In the uterus was present a small foetus (3 in.). Nervous system: brain was normal. Skeleton: bone marrow of humerus was fatty, that of femur likewise and slightly haemorrhagic.

Pathological anatomical diagnosis.—Blood extravasations into subcutaneous tissues, oedema and haemorrhage of lymph glands; petechiae in trachea, blood extravasation into lungs and ecchymoses of pleura; oedema of both lungs; abscesses of liver; ecchymoses in peritoneum, extravasation in epicardium and endocardium. Fibrous adhesion of diaphragm to right lung, liver, and reticulum; foreign body in reticulum; gastritis.

Microscopical examination.—Liver: only a portion of the liver was affected. The central vein was much dilated and filled with blood, the vessels were gorged. Around the central vein the liver cells had disappeared. In the detritus nuclei were seen, the cytoplasm did not take the stain. The cells in the periphery were intact and took the stain well.

Diagnosis.—Necrosis of the liver cells. Stasis.

Experiment No. 2.—Feeding of Ragwort to heifer 3223. She was kept at Onderstepoort since December, 1913. From the 28th February, 1916, to the 5th April, 1916, she received daily 8 oz. of Ragwort. At the beginning it was noticed that the plant was not eaten well, and again towards the end of March when a slight loss of condition became noticeable. Feeding was discontinued on the 5th April, 1917, the animal was kept under observation until the end of October, 1917. Nothing happened.

Experiment No. 3.—Heifer 3604. Originally obtained from the Cape Province and was at Onderstepoort since the 23rd June, 1915. The heifer was fed from the 20th March until the 30th March, 1916, receiving daily 8 oz. of Ragwort ($5\frac{1}{2}$ lb.). On the 25th March, 1916, it was noted that the heifer was not feeding well and had lost condition; on the 29th of March she was not feeding at all, found down and unwilling to rise. She passed black liquid faeces mixed with blood. The temperature was 101.2° F. The heifer died during the night of the 31st.

Post-mortem.—Condition was somewhat poor. Rigor mortis was present. Visible mucous membranes were pale. Flesh was brownish red. Pleural cavities were normal. Diaphragm was convexly forwards. Peritoneal cavity was normal. Lymph: mandibulars were haemorrhagic; retropharyngeals hyperaemic; prepectorals oedematous. The tongue was normal. Pharynx contained some blood-stained mucus and the oesophagus some ingesta. Respiratory organs: some ingesta was found in larynx and trachea. Mucosa of trachea showed petechiae. The left lung was partially collapsed, on section oedematous, bloodless. Ingesta was also found in bronchi. Right lung on section was found hyperaemic, anterior lobe showed some emphysema. Pericardium was empty. Heart: coagulated blood present in both ventricles. Epicardium showed ecchymoses, left endocardium haemorrhagic infiltrations. Myocardium was normal. Pulmonary artery was normal. Intima of thoracic and abdominal aorta was normal. Liver on section was yellowish; lobules distinct, consistence somewhat firm; central stasis of lobules. Gall bladder was distended with greenish red bile and coagulated blood, mucosa showed ecchymoses. Spleen measured 41×14 cm., on section follicles found enlarged, trabeculae were not distinct, pulp soft. Kidneys: capsule was easily detached, on section slight

hyperaemia of medulla was noted. Stomach: omasum had dry contents. Rumen and reticulum were normal; in abomasum were blood-stained contents, mucosa was hyperaemic and ecchymosed. Small intestines: blood-stained ingesta and coagulated blood were present, the mucosa was diffusely haemorrhagic. Large intestines: contents as in small intestines, mucosa showed a patchy hyperaemia; mucosa of rectum likewise. Bladder showed ecchymoses and petechiae. Sexual organs: in uterus was found a foetus (30 in. long). Skeleton: bone marrow was fatty and a few haemorrhagic foci were present.

Pathological anatomical diagnosis.—Oedema and haemorrhage of lymph glands. Oedema of left and stasis of right lung. Emphysema. Petechiae of trachea; ecchymoses in epicardiums and endocardiums. Icterus and stasis of liver. Chole-cystitis haemorrhagica. Hyperaemia of kidneys. Gastritis and enteritis. Tumor splenis.

Microscopical examination of the liver.—There was a stasis in the central vein, that involved the central portion of about half of the lobule. Here the liver cells were not visible, they had disappeared. Fat stain showed but little fat in the cells surrounding the veins.

Diagnosis.—Stasis and atrophy of the central part of the lobule.

Susceptibility to Senecio poison.—The toxicity of the various species of *Senecio* apparently varies and not all animals are equally susceptible. In the experiment of Chase with *Senecio burchelli* one ox died after it had eaten 1 lb., a calf fed on small quantities lived at least two months before it had to be killed. In Robertson's feeding trials with *Senecio latifolius* one ox had eaten 112 oz. before it died and the second one 156 oz. One feeding test with *Senecio latifolius* undertaken in Mooi River gave negative results, the heifer had eaten 27½ lb. within a period of one hundred and six days. Of the three experiments in Onderstepoort with *Senecio latifolius* one proved to be unsuccessful, the animal having consumed about 19 lb. over a period of thirty-six days, whilst one heifer succumbed after she had eaten 1 lb. and a second after she had consumed 5½ lb., the former in two days and the latter in ten days. It would thus appear that not all cattle are equally susceptible to the same species of Ragwort.

Symptomatology of the Ragwort poisoning in cattle.—Course of the disease: it was in all cases a short one, except Chase's calf, in which it appeared that *Senecio* had produced a kind of cachexia with a liver induration, which in the absence of microscopical investigations cannot definitely be diagnosed. One of the animals in Onderstepoort gave practically no warning at all, it died during the night; the second one was losing condition for three days and was very ill on the fourth. Chase's animals were sick for one and two days before they died, and Robertson's one ox was sick for fourteen days and the second one for twenty-four hours. The symptoms in the acute cases were those of a diarrhoea accompanied by straining in the case of *Senecio burchelli* poisoning, so that the rectum even became everted. The faeces were black in the Onderstepoort case (3606) mixed with blood. The smell was offensive. In the case of Robertson the diarrhoea lasted for fourteen days, the animal finally being unable to rise.

The pathological anatomy of Ragwort poisoning in cattle.—In all cases of acute death the lesions in the stomach and intestines were most pronounced. In *Senecio burchelli* poisoning the folds of the abomasum were much thickened due to an infiltration of straw-coloured fluid into and under the mucosa, which itself showed extensive haemorrhagic infiltrations in the form of ecchymoses and injected blood-vessels or even large suffusions. Superficial ulcers were also present, emanating from the haemorrhagic effusions. The same picture was described in *Senecio latifolius* poisoning of the acute and subacute case. Here lesions in small and large intestines were pronounced. In both instances their contents were blood-stained, and even blood coagula were present, and the mucous membrane was thickened, infiltrated with blood, and fibrinous coagula were on the surface. Diffuse hyperaemia of the rectum was present. The liver was in all cases found affected. Chase described it in *Senecio burchelli* poisoning to be in a state of acute venous congestion, whereas Robertson described it in *Senecio latifolius* to be cirrhotic, hard, and firm, and in one case even small. In the Onderstepoort cases the liver had an abnormal colour to brown or yellow, the appearance of stasis was pronounced. The gall bladder in all cases showed extensive lesions. It was distended with bile of a green reddish colour and even coagulated blood was present. The mucosa of the gall bladder showed ecchymoses in the injected and thickened mucosa. The heart was also the seat of lesions. The epicardium showed ecchymoses as well as the endocardium. There was hyperaemia of the kidneys present in one instance and also ecchymoses

in the urinary bladder. The liver examined microscopically in the two Onderstepoort cases showed the following: stasis of the central veins, which were much dilated and gorged with red corpuscles, extending into the lobule involving about its central half, where the blood covered everything, so that the liver cells could no longer be made out. Actual destruction of liver cells had taken place in one instance (3518), where detritus, not taking the stain, was left, indicating a necrosis. But little fat could be traced by means of Sudan staining.

Pathological anatomical diagnosis.—Cholecystitis haemorrhagica, gastritis, enteritis haemorrhagica. Stasis in the portal system of the liver, atrophy and necrosis of the central liver cells, ecchymoses in the heart.

Comparison of Ragwort poisoning in horses and cattle.—The toxic principles act in cattle more quickly than in horses, in the latter the poison seems to act only when absolutely or relatively large quantities have been consumed and over a prolonged period. The pathological picture in both is not identical. In cattle the disease is more one of the stomach and intestines and to a much lesser degree one of the liver and apparantly not at all of the kidneys and the blood; in the horse the liver and the kidneys are the principal organs affected and also the blood in some cases, whilst the intestines show but little changes and not of such a severe nature as found in cattle. Ragwort poisoning in cattle is a disease affecting the digestive tracts mainly, and in horses it is a disease of the parenchymatous organs, the liver in particular and of the heart. It is thus evident that the disease caused by the Senecio in South Africa does not cause a true liver cirrhosis, neither in the horse nor in cattle.

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EXPLANATION OF PLATES.

(ACUTE LIVER-ATROPHY AND PARENCHYMATOUS HEPATITIS IN HORSES.)

Fig. 1. Section of liver of horse dead of acute Liver-atrophy. Magn. 2×.

The grey meshes represent the periphery of the lobules containing the proliferated bile-ducts. The dark areas within the meshes are the necrotic central portion of the lobules, they are slightly sunken, and the white meshes slightly raised as ridges. In some of the lobules the central vein is visible as a small dot. The specimen had a green colour.

Fig. 2. *Horse 40, Pietpotgietersrust.*

Around the vena centralis is a zone of comparatively intact liver cells; cytoplasm and nuclei staining rather pale. The intermediate portion is occupied by blood and but little liver structure is visible. The periphery consists of vacuolated pale liver cells, proliferated bile-ducts, and round cell infiltration. Magn. 33×.

Fig. 3. *Horse W. 3148.*

The central vein shows hyaline thickening. Of the adjacent liver cells only remnants are left with vacuoles. A fairly large zone of the periphery is occupied by multinuclear cells (budding bile-ducts) and round cells, and between them relatively intact liver cells. Magn. 30×.

Fig. 4. *Horse L. 771.*

The centre of the lobule is occupied by blood replacing the liver cells, traces of which are left as large vacuoles. The periphery consists of large multinuclear cells (budding bile-ducts), round cells. Magn. 30×.

Fig. 5. *Horse 9989.*

The lobule is mainly filled with detritus and remnants of liver cells with large vacuoles. Some of the central veins with a slightly thickened hyaline wall. In the periphery large multinuclear cells (budding bile-ducts) and between them round cell infiltration. Magn. 33×.

Fig. 6. *Horse 40, Pietpotgietersrust.*

No normal liver cells visible in the lobules, their place is occupied by detritus. In the periphery some proliferated bile-ducts and round cell infiltration. Magn. 25×.

Fig. 7. *Horse T. 1525.*

The lobule is practically wholly occupied by a fine detritus, in which, adjacent to the central vein, only remnants of liver cells are left. In the periphery multinuclear cells (budding bile-ducts) with round cell infiltration around them. Magn. 33×.

Fig. 8. *Horse 11499.*

Acute Liver-atrophy: formation of new bile-ducts in the periphery of the lobules is distinct, the centre of the lobule being necrotic. Central vein engorged with blood. Magn. 33×.

Fig. 9. Section of liver of horse dead of Senecio poisoning. Natural size.

Parenchymatous Hepatitis. The grey meshes indicate the periphery of the lobules consisting of proliferating bile-ducts, and within in places are visible the slightly sunken septa. The dark areas in the meshes are the comparatively intact central portion of the lobules.

Fig. 10. *Horse 10755.*

Senecio poisoning. A thickening of the septum by homogeneous substance, fibroblasts and round cells. In the septa and adjacent to it large multinuclear cells (budding bile-ducts) and large deformed liver cells. Magn. 50×.

Fig. 11. Horse 7298.

Senecio poisoning. The septa are thickened by infiltration with homogeneous substance and round cells and vacuolated liver cells. No budding bile-ducts can be seen. The centre of the lobule is chiefly occupied by blood and atrophied liver cells. Magn. 30 \times .

Fig. 12A. Horse 11057.

In the periphery of the lobule, liver cells interlocking with budding bile-ducts. Septa are thickened by connective tissue and round cells. Magn. 25 \times .

Fig. 12B. The dark blotches are fat and pigment which is very frequent in this section. The peripheral portion contains the proliferating bile-ducts. Magn. 30 \times .

Fig. 12c. Large multinuclear cells (budding bile-ducts) and alongside normal liver cells. Magn. 150 \times .

Fig. 13. Horse 10717 (20th December, 1916), suffering from acute Staggers. Fresh bruises on head.

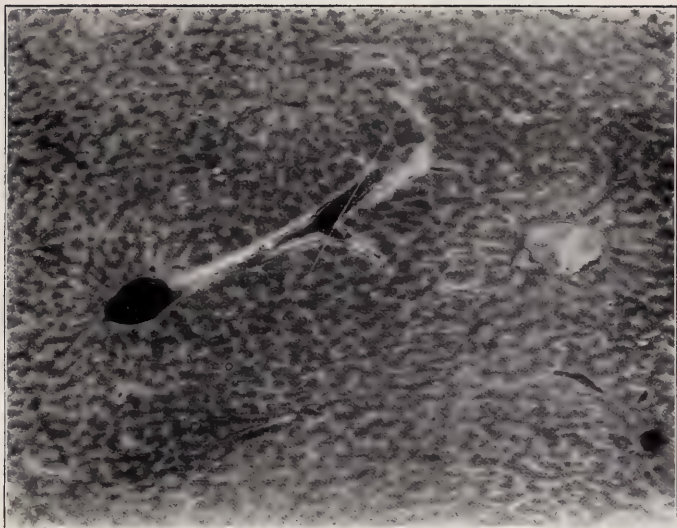


FIG. 1.

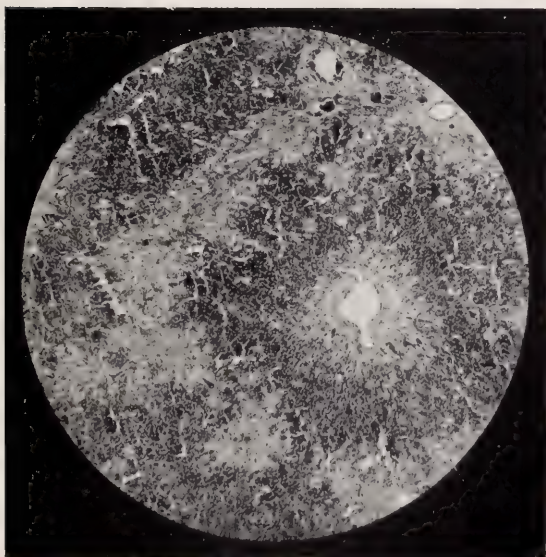


FIG. 2.

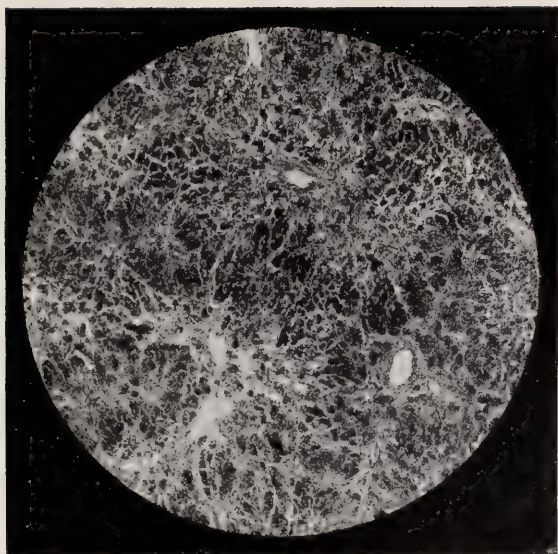


FIG. 3.

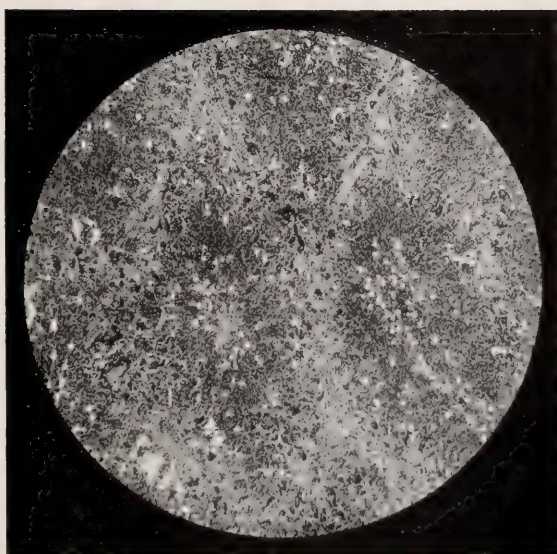


FIG. 4.

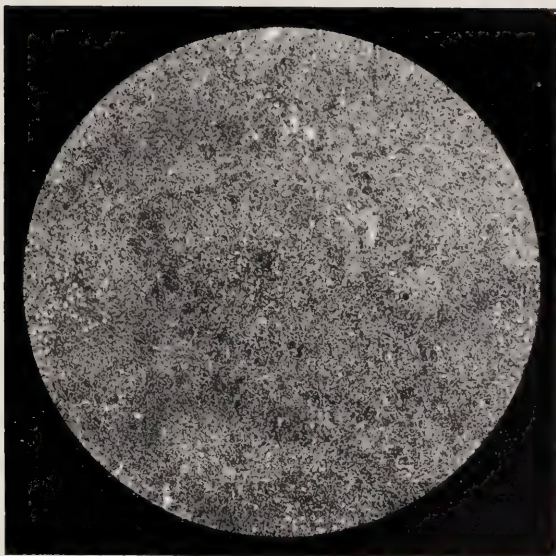


FIG. 5.



FIG. 6.



FIG. 7.

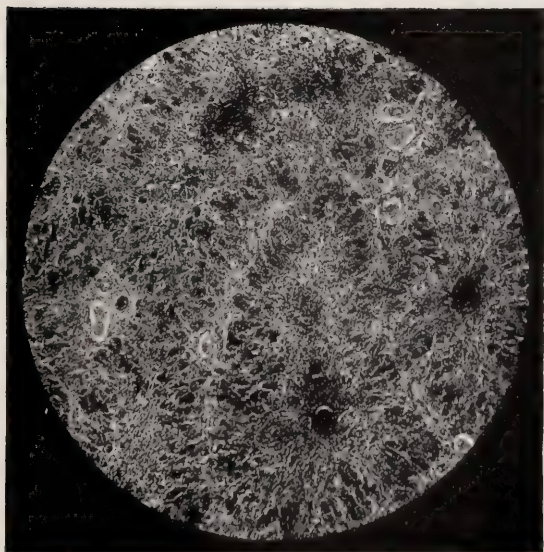


FIG. 8.



FIG. 9.

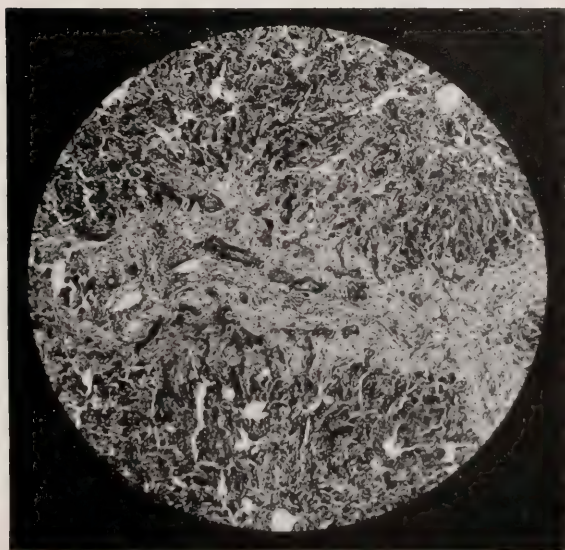


FIG. 10.

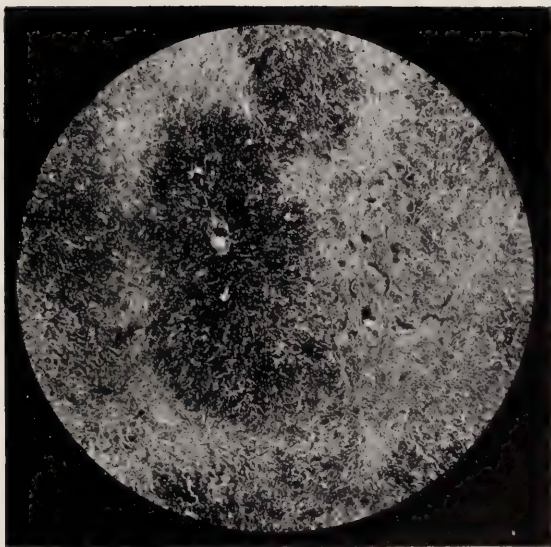


FIG. 11.

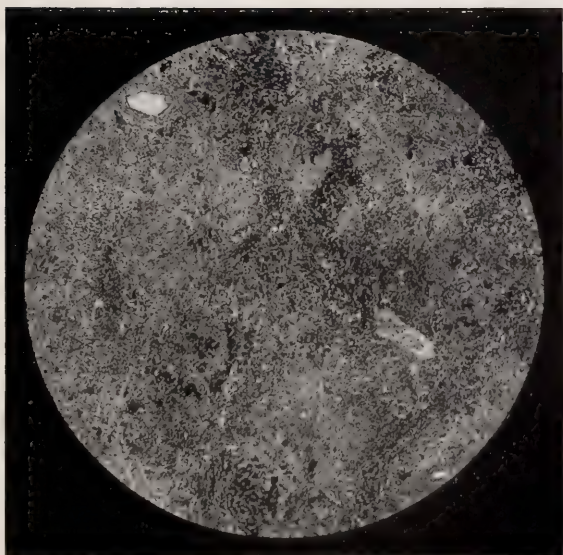


FIG. 12A.

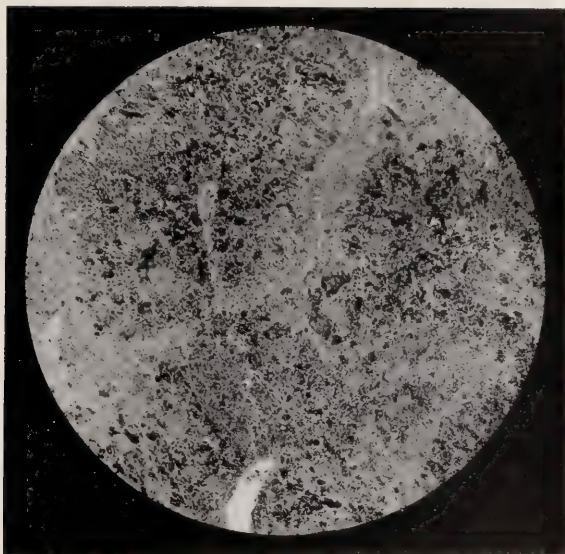


FIG. 12B.

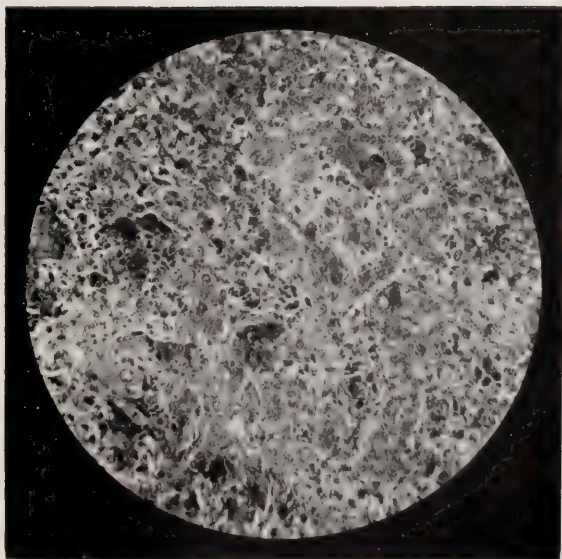


FIG. 12C.



FIG. 13.

Intestinal Invagination, Intussusception, in Sheep (Reckziekte or Knopderm).

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IN South Africa a pathological condition not infrequently occurs amongst sheep in which one portion of the intestine extends into a neighbouring part in such a manner that the former is contained within the latter, and, since a characteristic symptom is that the affected animal frequently places itself in a stretching position, it is known among farmers as "Reckziekte"; the term "Knopderm"—which has also been given to this condition—apparently originated from the resemblance of the affected part of the bowel to a knot.

Occurrence.—Reckziekte has been known in South Africa for a number of years; in fact, as far back as 1894* its occurrence was reported on some farms in the Britstown District of Cape Colony by Mr. Henning, Assistant Veterinary Surgeon of the Cape, who ascertained that, whereas in former times it was almost unknown in the district, the mortality on some farms during the years 1891 to 1894 was 5 per cent. to 10 per cent. of the total number of sheep farmed. In 1900† Hutcheon recorded his views as to the cause of Reckziekte.

At various times during the last few years a few cases have been observed at the Veterinary Research Laboratory, Pretoria, and it was fairly prevalent during the latter half of 1917 on some farms in the Cape and Orange Free State Provinces, the mortality on these varying between 0.75 per cent. to 7 per cent. of the total number of sheep farmed.

Age, sex, and condition of animals affected.—The experience of owners as to the age at which sheep become affected was found to differ; some encountered it mostly in young sheep (two-tooth), whilst others observed it in sheep of various ages up to eight-tooth.

The total number of cases which came under the writer's observation was 14; of these, one was a 15-months' old lamb, and the remainder either four, six, or eight tooth. Sheep of both sexes may become affected. Condition does not appear to be a determining factor, and sheep of good, fair, or indifferent condition may contract Reckziekte.

Etiology.—Reckziekte in sheep is generally associated with the nodules which are caused in the walls of the intestine as a result of

* Report of the Colonial Veterinary Surgeon and the Assistant Veterinary Surgeon for the year 1894, page 42.

† *Agricultural Journal*, Cape of Good Hope, 19th July, 1900, page 91.

parasitic invasion (*Oesophagostomum columbianum*). Hutecheon considered "that when the nodules became very numerous and closely packed all round the walls of the intestines they tend to paralyse that portion of the bowel. The muscular movement of the bowel being thus seriously affected the faeces are not moved onwards, and constipation intervenes. This leads to the animal straining considerably to force the faeces through the diseased and partially paralysed portion of the bowel. It is this straining which forces the portion of the bowel in front of the diseased portion into the latter, where it becomes fixed, and blocks up the passage completely."

Henning "suspected that the increase of Reckziekte in the Brits-town District was caused by the spread of *Oesophagostomum columbianum*, and this parasite was formerly entirely unknown there, and it seems not improbable that the little knots (nodules), which are produced by the larvae of this worm, facilitated the genesis of such invaginations of the ileum, and rendered also spontaneous reposition of the strangulated portions more difficult as the walls became uneven and rigid, and further, that in all cases which were examined, the walls of the ileum and caecum contained comparatively more knots (nodules) than any other part of the small and large intestines, and this might be the reason why the invagination invariably takes place on the above-described place."

In the cases which came under the writer's observation parasitic nodules were found, without exception, to exist in the bowel walls to a greater or less extent.

In human medicine considerable attention has been given to the study of intussusception, and a great deal of discussion has been created over the mechanism which occasions this, as a result of which a distinction is drawn between the agonal and the ordinary pathological variety. In the former, which occurs during the agonal stage, and is distinguishable from the latter in being reducible, there being little or no hyperaemia or adhesions, violent peristaltic movements apparently play an important rôle, while in the case of the latter the explanation is usually to be found in some organic defect, which occasions a prolapse of a segment of the bowel, which is carried downwards into a segment beyond. In normal peristalsis the movements of the intestines have been found to consist of two parts,* which, commencing at a certain point, pass backwards in a definite sequence, and seem to combine to facilitate the movement onwards from segment to segment, the advanced area of constriction being preceded by an area in which the bowel becomes wider and shorter.

Various investigations of the etiology of intussusception in cases in which no antecedent pathological lesion of the bowel has been found have indicated that the primary condition is a spasm of the circular muscle of the bowel in some portion. This constricted area being swallowed by the segment of bowel immediately below is seized by the peristaltic movements of the part into which it extends, and is carried forward. The firmer attachment by a short mesentery of some portion of the intestine and abnormalities of the mesentery, by permitting the more ready invagination of one part of the bowel into another, tumours, adhesions, and tympany are considered *predisposing causes*.

* Bayliss and Starling, *Journal of Physiology*, 24.9.1899.

The study* of a large series of cases of intussusception in the human subject shows an absence of any definite cause in the larger proportion of cases. In a relatively small number some history of acute intestinal disturbances or of injuries, contractions of the bowel wall, violent shaking, and the like, are met with, whilst in rare instances parasites have been held responsible.

Symptoms: Duration and Course.—Although marked symptoms of abdominal pain, such as the animal throwing itself down suddenly, rolling and marked uneasiness, were not observed, the affected animal sometimes turns the head towards the flank, and more often places itself in a stretching position, the fore legs brought well forward, the neck extended, the hind legs extended backwards and the back hollowed. The animal is disinclined to move, and either stands or lies; the pulse is accelerated; in some instances a rise of temperature was observed. The character of the evacuations was found to be of some importance from a diagnostic point of view. There is at first a discharge of faecal matter from the bowel; later this becomes less in quantity and harder, and should the movements continue the discharge becomes either tarry-like in consistence and mixed with blood, or it becomes liquid and mixed with mucus, or mucus and blood, while the discharge of faecal matter ceases. Sometimes haemorrhage from the bowel may occur. The wool round the tail is sometimes matted with discharges from the bowels, or there may be some hard, dry lumps containing blood, and dark red in colour, adherent to the wool below the tail. In some cases which recovered a loss of wool resulted. Recovery does not often occur. The affection usually ends in death in from four to fourteen days after symptoms are observed.

Pathological Anatomy.—The portion of the bowel within the ensheathing portion is known as the “intussusceptum,” and the ensheathing portion is known as the “intussusciens.” A transverse section through an intussusception shows three layers of bowel, viz., (1) the outer or ensheathing, which has the serous surface directed outwards as under normal conditions; (2) internal to this the returning layer, which has the mucous surface directed outwards, the mucous surfaces of the returning layer, and of the ensheathing layer being thus in contact; (3) internal to the returning layer is the entering layer, which has its serous surface outwards as normally, and in contact with the serous surface of the returning layer. The term “neck” is applied to that portion of the intussusception where the intussusceptum passes within the ensheathing bowel. The doubling of the bowel where the intussusciens joins the returning layer is known as the “collar.” When a complete intussusception occurs the entering portion draws along its mesentery with the blood-vessels of the latter, which will be subjected to a certain amount of pressure according to the degree of constriction at the neck. At first it may be possible to pull the entering layer out of the receiving layer, and thus reduce the intussusception, but when the malposition has been in existence for a short time, the obstruction of the mesenteric vessels leads to a congestion of the vessels of the intussusceptum and its mesentery; the thin-walled mesenteric veins entering the invaginated part are occluded by the pressure sooner

* Osler and McRae, Vol. 5, page 460.

than the arteries, so that the intussusceptum becomes swollen, oedematous, and through infiltration with blood, purplish or dark red in colour. The lumen of the bowel becomes partly or wholly obliterated with extravasated blood; some of the extravasated fluid may also escape into the peritoneal cavity. As a result of the passive congestion and obstruction of the lumen convulsive contractions may occur leading to an increase of the portion invaginated towards the anus, the posterior section slipping over the former. Peritonitis affecting the serous coats of the entering and returning layers of the bowel where they lie in contact sets in rather promptly, and may be confined to the region of the neck of the invagination, or may involve the entire serous covering within the sac and result in adhesions of these surfaces. The natural method of cure, viz., the formation of adhesions at the neck and sloughing or gangrene of the included portion of the bowel rarely occurs, the usual termination being obstruction of the bowels and death.

Location.—For a distance of about 25 cm. from the ileo-caecal valve the mesentery which envelops the ileum passes on to the caecum; the preceding portion of the ileum for a distance of about 180 cm. has a comparatively loose mode of suspension, the greater portion entering into the formation of a U-shaped loop. In the cases examined by the writer the intussusception was found to occur at a distance of from 23 to 164 cm. from the ileo-caecal valve, namely, in that portion of the ileum in which, owing to its looser attachment, movements were less restricted. (Fig. 9.) The following are the results of the examination made by the writer of a number of specimens of intussusception, which were either collected from cases which occurred at the Veterinary Research Laboratory or on farms:—

Specimen No. 1 (figs. 1 and 2).—The intussusception occurs in the ileum 88 cm. from the ileo-caecal valve, and extends in an antero-posterior direction for a distance of 34.6 cm. It has the following shape: it first makes a half-circle, then turns at an angle of 90° to form another half-circle, and turns again at an angle of 90° to form a third half-circle, half-circles 1 and 3 being parallel. The following are the measurements along its convexity: from neck to centre of first angle, 8.4 cm.; from centre of first angle to centre of second angle, 8.5 cm.; from centre of second angle to centre of third angle, 8.5 cm.; from centre of third angle to termination, 9.2 cm. The intussusception is irreducible and constricted at the neck. There are three parasitic nodules in the wall of the intussusciens, varying in size from .15 to .5 of a cm. The specimen includes anteriorly and posteriorly a portion of the ileum. The former measures 22 cm. in length, and in its wall there are six parasitic nodules; the latter measures 30 cm. in length; fourteen parasitic nodules, varying in size from .15 to .75 of a cm., occur in its wall. The intussusceptum is firm and sausage-like in consistency anteriorly; posteriorly it is softer. It is dark red in colour, and on section the following parasitic nodules are visible:—(1) Ten cm. from its extremity in the wall of the entering portion of the bowel; size of nodule .75 cm. in diameter. (2) Fifteen cm. from its extremity in the wall of the entering portion of the bowel; size .5 cm. in diameter. (3) Three cm. from its extremity in the wall of the returning portion of the bowel; size .5 cm. in diameter. (4) At the neck in the wall of the entering portion of the bowel; size .5 cm. in diameter. The intussusceptum

includes the mesentery of the enfolded bowel; the walls of the entering and returning portions are infiltrated and thickened. The lumen of the bowel is obliterated and contains a haemorrhagic exudate. The serous layers of the entering and returning portions of the bowel, where they are not in contact, are adherent consequent on the peritonitis.

Specimen No. 2 (figs. 3 and 4).—The intussusception occurs in the ileum 30 cm. from the ileo-caecal valve, and extends in an antero-posterior direction (an anterior section of the bowel having become invaginated into the immediately posterior section). It has the shape of a spiral, and measures over its convexity from neck to termination 14 cm. The intussusception is irreducible and constricted at its neck. The specimen includes anteriorly and posteriorly a portion of the ileum; the former measures 10 cm. in length; in its wall are three parasitic nodules, situated $1\frac{1}{2}$, 5, and 9 cm. respectively from the neck; the latter portion measures 48 cm.; nine parasitic nodules occur in its wall. The diameter of the above-mentioned nodules varies from .2 to .5 cm. in diameter. The intussusceptum is firm, sausage-like in consistency and dark red in colour. On horizontal section a greenish caseous nodule is visible in the wall of the returning portion of the bowel, $1\frac{1}{2}$ cm. posterior to the collar; size .5 cm. in diameter. At the neck in the wall of the entering portion of the bowel there are two nodules. The walls of the entering and returning portions of the bowel are infiltrated and thickened. The lumen of the bowel is obliterated and contains a dark red coagulated haemorrhagic exudate. The serous layers of the enfolded portions of the bowel are adherent as a result of the peritonitis. The intussusceptum includes the mesentery of the enfolded bowel.

Specimen No. 3 (fig. 5).—The intussusception occurs in the ileum 23 cm. from the ileo-caecal valve, and has the following shape: it forms a half-circle, then turns at an angle of 90° to form a second half-circle, and turns again at an angle of 90° to form another half-circle; half-circles 1 and 3 are thus parallel. It has the following measurements over its convexity: from neck to centre of first angle, 5 cm.; from neck to centre of second angle, 15.5 cm.; from centre of second angle to termination of intussusception, 16 cm. It is irreducible and constricted at its neck. The specimen includes anteriorly and posteriorly a portion of the ileum; the former measures 25 cm. in length, and contains several nodules in its walls; the latter portion also contains several nodules in its walls. The intussusciens is bluish in colour, and its vessels are well mapped out. In the wall of the intussusciens there are six nodules, varying in size from .5 to .75 cm. in diameter. Fibrous filaments occur on the serosa of the intussusciens, and on the serosa covering the nodules. Fibrous adhesions exist at the neck between the serosa of the returning and entering portions of the bowel. The intussusceptum is firm and sausage-like in consistency, swollen, and dark red in colour. On horizontal section nodules are found in the following positions:—(1) In the wall of the entering portion of the bowel there is a nodule .5 cm. in diameter, greenish and caseous on section. (2) At the termination of the intussusceptum in the wall of the entering portion of the bowel there is a nodule 0.5 cm. in diameter. (3) Three nodules situated at various distances from the neck in the wall of the

entering portion of the bowel. The walls of the enfolded portion of the bowel are dark red in colour and infiltrated with haemorrhagic exudate. The lumen of the bowel contains a haemorrhagic exudate. The serosa of the bowel are not adherent except at the neck.

Specimen No. 4 (figs. 6 and 7).—The intussusception occurs in the ileum and extends in an antero-posterior direction, a portion of the bowel being invaginated into the immediately posterior section. The intussusception is irreducible and constricted at the neck, and has the following shape: it makes a half-circle, and turns at an angle of 180° to form another half-circle parallel to the first. It measures 11 cm. from the neck to the centre of the angle, and 11 cm. from the centre of the angle to its termination. The specimen includes anteriorly a portion of the ileum, measuring 10 cm. in length; in the wall of this there are several nodules. The intussusceptum is dark red in colour and firm and sausage-like in consistency. The walls of the enfolded portion of the bowel are thickened. The lumen of the bowel is obliterated and contains a haemorrhagic exudate. A haemorrhagic exudate occurs between the intussusciens and the returning layer of the intussusceptum. In the neck in the wall of the entering portion of the bowel there is a parasitic nodule .75 cm. in diameter.

Histo-Pathology.—Fig. 7 is a transverse section through the wall of the intussusception at a distance of 5 cm. from its termination. The measurement from the external part of the intussusciens to the lumen of the entering portion of the bowel (where no mesentery is included) is 7 mm.; when the measurement is included, 13 mm. The nuclei of the muscle fibres of the outer longitudinal and inner circular coat of the intussusciens are distinct. The villi of the mucosa of the intussusciens with their epithelial covering stain distinctly. The villi of the returning and entering portion of the bowel with their epithelium do not stain. The nuclei of the muscular fibres of the returning and entering bowel are unstained. The mesentery is drawn in between the entering and returning layers of the bowel. The walls of the entering and returning layers of the bowel are infiltrated and thickened. The lumen of the bowel is obliterated, and contains a haemorrhagic exudate.

Specimen No. 5 (Sheep No. 12505; post-mortem No. 12180, 23rd October, 1917).—The intussusception occurs 30 cm. from the ileo-caecal valve, a portion of the bowel being invaginated into the immediately posterior section. It has the following shape: it first makes a half-circle, then turns at an angle of 90° to make another half-circle, and turns again at an angle of 90° to make a third half-circle, and measures along its convexity 26 cm. The specimen includes anteriorly and posteriorly a portion of the ileum; the former measures 30 cm. in length. Several parasitic nodules occur in its walls; the first is about 10 cm. from the neck; the latter portion is 30 cm. in length, and there are several parasitic nodules in its walls. There is also a large nodule in the walls of the ileo-caecal valve. In the walls of the intussusciens there are two nodules. The intussusceptum is dark red in colour, and firm and sausage-like in consistency anteriorly, fluctuating posteriorly. On horizontal section the following nodules are visible:—(1) In the neck in the wall of the entering portion of the bowel there is a greenish-coloured parasitic nodule. (2) In the wall of the entering portion of the bowel—midway

between the neck and its termination—there is a nodule .25 cm. in diameter. The lumen of the bowel contains anteriorly an exudate of coagulated blood, and posteriorly a fibrinous exudate. The serosa of the enfolded portions of the bowel are adherent. Fibrous filaments occur in the serosa of the intussusciptions. The intussusceptum includes the mesentery of the enfolded bowel.

Post-mortem on Sheep No. 12505 (post-mortem No. 12180, 23rd October, 1917).—Merino hamel, six-tooth. Interim: a few hours. Condition: rather poor. Natural openings: rectum slightly everted; mucus and some ingesta from nostrils. Eyes: sunken. Conjunctival mucous membrane injected. Flesh: normal. Subcutaneous tissues: fat sparing. Lymph glands: mandibulars normal; postpharyngeals normal. Tongue: normal. Oesophagus: normal. Trachea: some foam; mucous membrane normal. Peritoneal cavity: a blood-coloured exudate. Respiratory organs: lungs, marked hyperaemia and oedema; ingesta in bronchi. Circulatory organs: heart in systole, ventricles almost empty. Epicardium: ecchymoses. Endocardium: normal. Myocardium: normal. Liver: rather soft; rich in blood; on section lobules distinct. Periportal lymph glands: hyperaemic. Gall bladder: small quantity of dark-green coloured bile. Mucous membrane of gall bladder: bile stained. Spleen: 10×6 cm. Greatest depth 2 cm.; on section, normal. Kidneys: fat, sparing in adipose capsule; capsule easily detached; consistence firm; on section hyperaemic. Suprarenal glands: normal. Abomasum: contents pea-soup consistence; mucous membrane swollen and hyperaemic; sub-mucosa infiltrated; no wire-worm (*Haemonchus contortus*) present. Omasum, reticulum, and rumen: contents partly liquid; mucous membrane normal. Small intestines.—Duodenum: mucous membrane hyperaemic. Jejunum: mucous membrane hyperaemic in patches; contents thin in consistence; much liquid in parts; contents mixed with blood. Ileum: about 30 cm. from the ileo-caecal valve there is an intussusception measuring 26 cm. over its convexity. Mucous membrane of ileum: hyperaemia in parts; parasitic nodules fairly frequent in the walls of the small intestines. Caecum: parasitic nodules in walls; mucous membrane normal; a small amount of contents. Large intestines: parasitic nodules frequent in walls; mucous membrane normal; mucus contents in strings and a small quantity of faecal matter. Bladder: urine clear; amber colour; mucous membrane of bladder normal. Rectum: patchy hyperaemia. Pathological anatomical diagnosis: hyperaemia and oedema pulmonum; ecchymoses epicardii; invaginato intestinalis. Cause of death: intussusception of the ileum (Reckziekte).

Specimen No. 6 (subject: 6-tooth Merino Hamel No. 12688, 13th December, 1917).—The intussusception occurs in the ileum 115 cm. from the ileo-caecal valve, and extends in an antero-posterior direction (a portion of the bowel having become invaginated into the immediately posterior section). It has the following shape: it first makes a half-circle at an angle of 90° , then turns to make another half-circle at an angle of 90° , and turns again to make another half-circle. It measures 23 cm. along its convexity. The intussusception is irreducible and constricted at the neck. In the walls of the bowel anterior to the intussusception there are less than one dozen nodules, and in the walls of the bowel posterior to the intussusception there are

six nodules. The intussusceptum is dark red in colour, firm and sausage-like in consistence anteriorly, and soft and fluctuating on pressure posteriorly. On horizontal section the following nodules are visible:—(1) At the neck in the wall of the entering portion of the bowel and extending into the lumen there is a nodule .75 cm. in diameter. (2) About midway between the neck and the termination of the intussusceptum in the wall of the entering portion of the bowel there is a nodule .5 cm. in diameter. The intussusceptum includes the mesentery of the invaginated bowel. The walls of the entering and returning portions of the bowel are infiltrated with a haemorrhagic fluid. The lumen of the bowel contains a haemorrhagic exudate, and is partly obliterated. The serous layers of the enfolded portions of the bowel are adherent.

Specimen No. 7.—The intussusception occurs in the ileum at a distance of 38 cm. from the ileo-caecal valve and extends in an antero-posterior direction. It has the shape of a half-circle, and measures over its convexity from the neck to its termination 15 cm. The intussusception is irreducible owing to congestion and consequent swelling of the intussusceptum. Nodules occur not frequently in the walls of the bowel anterior to the intussusception. There is one nodule 12 cm. and another 40 cm. from the neck. No others occur up to within 44 cm. from the neck. In the portion of the bowel between the termination of the intussusception and the ileo-caecal valve there are thirteen nodules. Nodules are present in the valvula ileo caecalis. In the walls of the intussusciens there are five nodules; one 3 cm. from the neck, two between the neck and the termination, and two at the termination of the intussusciens. The intussusciens is softish in consistency. The entering portion of the bowel is dark red in colour, and infiltrated with a haemorrhagic exudate. The serosa of the entering layer is adherent to the serosa of the returning layer. The following parasitic nodules occur:—(1) In the wall of the entering part of the bowel at the neck there is a nodule .75 cm. in diameter. (2) In the wall of the returning portion of the bowel, 5 cm. from the neck on the opposite side to the above, there is a nodule .5 cm. in diameter. Some adhesions exist between the serosa of the returning and entering layers at the neck. The mucosa of the portion of the bowel between the termination of the intussusception and the ileo-caecal valve is hyperaemic and contains blood.

Post-mortem on Sheep No. 13635 (post-mortem No. 12386, 21st January, 1918).—6-tooth Merino hamel. Condition: fair. Interim: 2-3 hours. Natural openings: normal; visible mucous membranes injected; flesh dark in colour. Subcutaneous tissues: fair amount of fat. Mandibular lymph glands: haemorrhagic. Pharyngeal lymph glands: haemorrhagic; tongue normal. Cervical trachea: slight hyperaemia of larynx; oesophagus normal. Peritoneal cavity: fair quantity reddish yellow turbid liquid. Pleural cavities: little or no liquid. Respiratory organs: left lung collapsed; pleura smooth; on section marked hyperaemia. Bronchus: vessels injected. Thoracic trachea: vessels injected. Thoracic oesophagus: normal. Thoracic aorta: normal. Pericardium: little or no fluid. Circulatory organs.—Epicardium: normal. Right ventricle and auricle: dark blood coagulum. Right endocardium: normal; valves normal. Left

ventricle and auricle: dark blood coagulum. Left endocardium and valves: normal. Myocardium: dark in colour; consistence normal. Liver: capsule normal; lobules distinct; colour of liver pale yellow brown; consistence soft; dark green turbid bile in gall bladder. Spleen: 9×7 cm.; greatest depth 1 cm.; capsule normal; on section trabeculae distinct; colour and consistence normal. Stomach.—Abomasum: slight hyperaemia of mucous membrane; omasum normal; reticulum normal; rumen normal. Small intestines: mucous membrane slight hyperaemia; about 30 cm. from the ileo-caecal valve there is an intussusception measuring over its convexity 15 cm.; parasitic nodules frequent in walls of small intestines; portion of the bowel between the intussusception and the ileo-caecal valve contains blood. Mucous membrane: hyperaemia. Caecum: parasitic nodules in walls; mucous membrane normal; rectum normal. Bladder: pale yellow-brown turbid urine. Pathological anatomical diagnosis: hyperaemia of lungs; fatty degeneration of liver; invagination intestini; parasitic infection. Cause of death: invagination of the ileum (Reckziekte).

Specimen No. 8 (Sheep No. 13731).—The intussusception occurs in the ileum 164 cm. from the ileo-caecal valve, and extends in an antero-posterior direction. It is a half-circle in shape and measures over its convexity 14 cm. The intussusception is reducible. Parasitic nodules are fairly frequent in the walls of the small and large bowels. In the length of bowel anterior to the intussusception, and which measures 25 cm., there are eight nodules. In the length of bowel between the termination of the intussusception and valvula ileo caecalis there are 60 nodules. Three nodules occur in the valvula ileo caecalis. In the wall of the intussusciptens there are five nodules in the following positions: one at the commencement of the invagination; the others at varying distances between the neck and termination. The intussusceptum is softish in consistency and on section reddish in colour. There is some liquid blood in the lumen, and some liquid blood between the entering and the returning walls, the walls of which are infiltrated with blood. The following parasitic nodules occur:—(1) In the wall of the entering portion of the bowel at the neck there is a caseous green nodule approximately 1 cm. in diameter. (2) In the wall of the entering bowel close to its termination there are two nodules. The intussusceptum includes the mesentery of the enfolded bowel.

Post-mortem on Sheep No. 13731 (post-mortem No. 12394, 23rd January, 1918).—Merino hamel. Age: six-tooth. Interim: about 12 hours. Condition: rather poor; abdomen distended. Natural openings: anus normal; nostrils normal; visible mucous membranes pale; blood normal in appearance; flesh rather pale; post-mortem changes. Subcutaneous tissues: fair amount of fat. Lymph glands: mandibulars and pharyngeals normal; tongue normal; oesophagus normal. Peritoneal cavity contains about 300 c.c. of blood-coloured liquid; diaphragm convexly forward; pleural cavities little or no liquid. Respiratory organs.—Left lung: hypostasis; hyperaemia and oedema; anterior lobe emphysema. Right lung: hyperaemia and slight oedema. Cervical trachea: mucous membrane reddish in colour. Thoracic trachea: near bifurcation some foam mixed with blood. Pericardium: some blood-coloured liquid; right heart in

diastole; left heart in systole. Right ventricle: dark blood coagulum. Left ventricle: almost empty. Left endocardium: slight ecchymoses. Right endocardium: imbibition. Epicardium: ecchymoses along transverse and longitudinal furrows. Liver: pale; fatty; post-mortem changes. Spleen: size 10×8 cm.; greatest depth 2 cm.; pulp soft dark; trabeculae not distinct; post-mortem changes. Kidneys: post-mortem changes; both kidneys soft; easily broken down on pressure; capsule easily strips. Stomach (fourth stomach): diffuse hyperaemia; contents liquid. Omasum, reticulum, rumen: contents pea-soup consistency; mucous membrane normal. Small intestines.—Duodenum: mucous membrane hyperaemic. Jejunum; parasitic nodules fairly frequent in walls; mucous membrane pale. Ileum: some liquid contents in bowel; parasitic nodules fairly frequent in its walls. At a distance of 164 cm. from the ileo-caecal valve there is an intussusception measuring over its convexity 14 cm.; little or no contents in ileum posterior to the invagination; mucous membrane of ileum posterior to the invagination hyperaemic. Caecum: parasitic nodules in walls; mucous membrane normal; contents small in quantity and normal consistence. Colon: parasitic nodules in walls; mucous membrane normal; contents small in quantity and normal in consistence. Rectum: normal; faeces in pellets rather harder than normal. Mesentery: fat sparing. Bladder: urine clear, pale-yellowish in colour; mucous membrane of bladder normal. Pathological anatomical diagnosis: post-mortem changes; gastritis; hyperaemia and oedema pulmonum; invaginatio intestini; parasitic infection. Cause of death: intussusception of ileum (Reckziekte).

Specimen No. 9 (Merino Ewe, about 15 months old).—History of case: was noted sick for six days previous to date of death. Symptoms: animal occasionally placed itself in a stretching position; off feed; moved about very little, either standing or lying; faeces mixed with blood; on the wool round anus hard lumps, dark red in colour, which on examination were found to be principally composed of blood. On the 5th evening temperature 104° F.; respirations, 40; lips and gums pale; conjunctival mucous membrane pale; on the morning of the 6th temperature 105° F.; respirations, 24; lying down; no inclination to rise when disturbed; not feeding; condition fair. Post-mortem immediately after slaughter. Integument: normal. Natural openings: some blood in hard cakes matted to wool round anus. Blood: normal in appearance. Flesh: rather moist. Peritoneal cavity: contains a lot of clear, watery-like fluid, which coagulates in the form of a whitish jelly-like mass shortly after opening into cavity. Trachea: mucous membrane normal. Lungs: normal. Heart: about 30 c.c. of clear watery-like fluid in pericardium. Endocardium: normal. Myocardium: normal. Liver, kidneys, spleen: normal. Abomasum: mucous membrane normal. Haemonchus contortus rare; contents liquid. Omasum: mucous membrane normal. Reticulum and rumen: mucous membrane normal. Small quantity of ingesta in rumen. Duodenum: mucous membrane normal. Jejunum: mucous membrane normal; contains an abnormal amount of liquid; turbid; parasitic nodules in walls. Ileum: contains a considerable quantity of liquid anterior to the intussusception; mucous membrane diffuse hyperaemic; 67 cm. from the ileo-caecal valve a portion of the ileum invaginated into the immediately posterior section for a distance of 20 cm.; vessels of

intussusception well mapped out; intussusceptum dark red in colour and fluctuating; intussusception irreducible, constricted at neck; adhesions between the serosa of intussusciens and intussusceptum at the neck; parasitic nodules in ileum, in walls of the intussusceptum; one nodule in wall of entering bowel at neck. Caecum: mucous membrane normal; almost empty; contents adhering to wall; black in colour; mixed with blood; tar-like; mucous membrane normal; parasitic nodules in walls of caecum. Colon: mucous membrane normal; contents similar in appearance and consistency to that seen in the caecum; parasitic nodules in walls of colon. Rectum: mucous membrane normal; contents similar to that seen in colon. Small intestines and abomasum examined for parasites (nematodes) with negative results. Pathological anatomical diagnosis: slight hydropericardium; ascites; invagination intestini; parasitic infection. Cause of death: killed; intussusception (Reckziekte).

Specimen No. 10.—The intussusception occurs in the ileum 56 cm. from the ileo-caecal valve, a portion of the bowel being invaginated into the immediately posterior section. It has the following shape: it forms a half-circle, turns at an angle of 90° to form a second half-circle, and turns again at an angle of 90° to form a third half-circle. It measures 32 cm. over its convexity. It is irreducible and constricted at its neck. The specimen includes anteriorly and posteriorly a portion of the ileum. The former measures 56 cm. in length, and contains in its walls five parasitic nodules varying in size from .25 to .5 cm. in diameter. The posterior portion measures 56 cm. in length, and contains 19 nodules in its walls. There are three nodules in the walls of the intussusciens, and there are some fibrous filaments on its serosa close to the neck. The intussusceptum is dark red in colour and includes the mesentery of the invaginated bowel. It is fluctuating in consistency. On horizontal section nodules occur in the following positions:—(1) At the neck of the wall of the entering portion of the bowel. (2) In the wall of the entering portion posterior to the neck. (3) In the wall of the returning portion of the bowel near its termination. The lumen of the bowel is not obliterated; it contains some haemorrhagic exudate. The walls of the enfolded bowel are infiltrated with a haemorrhagic exudate. The entering and returning portions of the bowel are not firmly adherent. In the posterior portion of the intussusceptum the lumen of the entering portion of the bowel contains a fibrinous exudate.

Specimen No. 11 (subject: Merino Hamel).—The intussusception occurs in the ileum 28 cm. from the ileo-caecal valve, a portion of the bowel being invaginated into the immediately posterior section. It is U-shaped and measures over its convexity 22 cm. in length. The intussusception is irreducible and constricted at its neck. The specimen includes anteriorly and posteriorly a portion of the ileum. The former measures 6 cm. in length; in its walls there are two nodules, situated 2 and 3 cm. respectively from the neck. The latter portion measures 8 cm. There are twelve parasitic nodules in its walls, varying in size from .5 to .75 cm. in diameter. In the walls of the intussusciens there are six nodules. Fibrous filaments occur on the serosa of the intussusciens, and form adhesions at the neck of the intussusceptum. The intussusceptum is dark red in colour, swollen, and sausage-like in consistency. On horizontal section the

following parasitic nodules are visible:—(1) In the neck in the wall of the entering portion of the bowel there is a nodule. (2) Two cm. from the above there is a nodule, also situated on the entering portion of the bowel. (3) About 5 cm. from the latter there are two nodules. (4) On the wall of the returning portion of the bowel at the neck there is a nodule. (5) In the wall of the returning portion of the bowel between the neck and its termination there are nine nodules. The lumen of the bowel is obliterated and contains dark red coagulated blood. The serosas of the entering and returning portion of the bowel are adherent. The mesentery of the enfolded bowel is included in the intussusceptum.

Specimen No. 12 (Sheep No. 11892, age 8-tooth).—The intussusception occurs in the ileum about 30 cm. from the ileo-caecal valve, a portion of the bowel being invaginated into the immediately posterior section. It has the shape of a half-circle and measures over its convexity from neck to termination 20 cm. in length. In the walls of the bowel anterior to the section parasitic nodules are fairly frequent, and in the portion between the termination of the intussusception and the ileo-caecal valve there are several nodules. Parasitic nodules are frequent in the walls of the caecum and colon. In the walls of the intussusciptiens there are several parasitic nodules. The intussusciptiens is bluish in colour, and the vessels in its walls are well mapped out. The intussusception is irreducible, constricted at the neck, and on section the intussusceptum is found to be dark red in colour and sausage-like in consistency. In the neck of the wall of the entering bowel there is a nodule about .75 cm., and in the walls of the returning layer there are three nodules near the collar, the others between the collar and the termination of the intussusceptum. The walls of the enfolded bowel are infiltrated with a haemorrhagic exudate; the lumen of the bowel contains a haemorrhagic exudate. There are adhesions between the serosa of the entering and returning layers of the enfolded bowel and adhesions at the neck of the intussusception.

Specimen No. 13 (Sheep No. 11663).—The intussusception occurs in the ileum about 25 cm. from the ileo-caecal valve, a portion of the ileum being invaginated into the immediately posterior section. It has the following shape: it first makes a half-circle and then turns at an angle of 180 degrees to form a second half-circle; length measured over its convexity, 27 cm.; parasitic nodules are fairly frequent in the walls of the bowel anterior and posterior to the section. In the walls of the intussusciptiens there are six parasitic nodules. The intussusception is irreducible and constricted at the neck. The intussusceptum is reddish in colour, firm in consistency, and on section the following nodules occur, viz., in the walls of the entering bowel there are three nodules, one at the neck and two close to its termination. In the walls of the returning bowel there is a nodule 32 cm. from the neck. The serosa of the entering and returning layers of the intussusceptum are adherent; some fibrous adhesions occur at the neck. The walls of the enfolded bowel are infiltrated with a haemorrhagic fluid, and the lumen of the bowel contains a haemorrhagic exudate.

Post-mortem on Merino Hamel No. 11663 (post-mortem No. 12087).—Age, 8-tooth. Condition, fair; abdomen, slightly distended; interim, one hour; rigor mortis not complete; natural openings, normal; visible mucous membranes, normal. Blood: normal in appearance; flesh,

normal. Subcutaneous tissues: fair amount of fat. Lymph glands, bronchial and mediastinal: normal; tongue, normal; oesophagus contains some ingesta; mucous membrane, normal. Peritoneal cavity: fibrinous exudate on serosa of bowels and on posterior surface of the liver and diaphragm; gastric omentum, two hydatids. Respiratory organs: lungs, slight hyperaemia. Trachea: a little foam at bifurcation; mucous membrane of trachea normal; pericardium almost empty. Circulatory organs: both ventricles contain coagulated blood; endocardiums, normal; myocardium, normal; epicardium, normal; fair amount of fat at base of heart; liver, normal; lobulations, distinct. Gall bladder: mucosa thickened; fibrinous deposit on wall of gall bladder; bile, dark ochre colour, consistence of treacle. Spleen: 10×7 cm., greatest depth 1.75 cm.; pulp, normal; trabeculae, distinct. Kidneys: left smaller than right; left 5 cm. in length; right 6.5 cm. in length; capsule easily detached; on section kidneys normal. Abomasum, omasum, reticulum: normal. Rumen: mucous membrane normal; contents consistence of pea-soup. Duodenum: mucous membrane normal. Jejunum: mucous membrane normal; parasitic nodules fairly frequent. Ileum: a portion of the bowel about 25 cm. from the ileo-caecal valve invaginated for a length of about 7 cm. Caecum: faeces in hard, dry pellets; parasitic nodules frequent in walls of caecum; mucous membrane normal. Colon: parasitic nodules fairly frequent in walls of colon; faeces in hard, dry pellets; mucous membrane normal. Contents of the jejunum, ileum, and caecum examined for microscopic nematodes; result negative. Pathological anatomical diagnosis: peritonitis; invaginatio intestini. Cause of death: intussusception of the ileum (Reckziekte).

Specimen No. 14 (Sheep No. 13763, 6-tooth Merino Hamel; post-mortem No. 12404).—The intussusception occurs in the ileum about 25 cm. from the ileo-caecal valve, and it has the following shape: it first makes a half-circle and turns at an angle to form a second half-circle; it measures along its convexity 33 cm.; portion of the anterior bowel measures 14 cm.; it has three nodules in its wall $5\frac{1}{2}$ cm. from the neck; portion of the bowel posterior measures 5 cm.; it has two nodules in its walls. In the intussusciens there are four visible nodules, two at the termination of the intussusciens, one about 12 cm. from the termination, and another about 10 cm. from the termination, irreducible. When the intussusciens is incised some bloody liquid escapes from the termination. The anterior portion of the intussusceptum is firm and sausage-like in consistency; the posterior part soft and fluctuating and dark red in colour. In the wall of the returning part of the bowel, 11 cm. from the termination of the intussusceptum, there are two nodules and in the neck of the entering wall one nodule.

SUMMARY.

(1) Intussusception of the ileum occurs, but not to any considerable extent, amongst sheep in South Africa. (2) It is commonest during a wet season, when *Oesophogostomum columbianum* infection is most prevalent. (3) Cases were observed in sheep of various ages, from 15 months upwards, and in animals in good and fair condition. (4) Intussusception occurred amongst sheep grazing under natural

conditions as well as amongst sheep which had been housed and artificially fed for varying periods; shortest period, one week; longest period, several weeks. (5) In the cases which came under the writer's observation the intussusception occurred where the movements of the bowel were less restricted, namely, in the terminal portion of the ileum at distances varying from 23 to 164 cm. from the ileo-caecal valve. (6) Parasitic nodules which originated from infection with *Oesophogostomum columbianum* parasites were always found in the wall of the small intestine, the caecum, and the colon in sheep affected with intussusception. (7) Parasitic nodules were found in the walls of the intussusciptens and in the walls of the enfolded portion of the intussusceptum, and usually in the wall of the entering or returning portion of the intussusceptum, either in or close to the neck. (8) The nodules may cause some constriction to the bowel wall (fig. 8). (9) In some cases fibrous filaments were formed on the serosa covering the nodules as a result of the inflammatory action set up by the presence of the nodules in the bowel wall. (10) In normal peristalsis an anterior section of the bowel becomes contracted and a portion of this passes momentarily within the immediately posterior section, and returns to its former position, where it remains until the succeeding wave occurs. This movement may cause a nodule, particularly in those cases in which the nodule causes a contraction of the wall (fig. 8), to come in contact with the serosa of the immediately posterior section, and cause a roughening of it, resulting in adhesions between the nodules and the serosa consequent on the peritonitis set up; or the peritonitis and adhesions may result from the presence of the nodule itself. In this way an anterior section may become fixed, and originate an intussusception; the anterior section becoming fixed, and prevented from returning to its normal position, is ensheathed by the immediately posterior section.

EXPLANATION OF PLATES.

Arrows indicate parasitic nodules.

Fig. 1.—Intussusception of ileum.

Fig. 2.—Section through same.

Fig. 3.—Intussusception of ileum.

Fig. 4.—Section through same.

Fig. 5.—Intussusception of ileum, showing fibrous filaments.

Fig. 6.—Section through intussusception of ileum.

Fig. 7.—*a* 1.—Serosal coat of the intussusciens not present in the section.

a 2.—Serosal coat of the returning portion of the bowel.

a 3.—Serosal coat of the entering portion of the bowel.

b 1.—Outer or longitudinal muscular coat of the intussusciens.

b 2.—Outer or longitudinal muscular coat of the returning portion of the bowel.

b 3.—Outer or longitudinal muscular coat of the entering portion of the bowel.

c 1.—Inner or circular muscular coat of the intussusciens.

c 2.—Inner or circular muscular coat of the returning portion of the bowel.

c 3.—Inner or circular muscular coat of the entering portion of the bowel.

d 1.—Submucosa of the intussusciens.

d 2.—Submucosa of the returning portion of the bowel.

d 3.—Submucosa of the entering portion of the bowel.

e 1.—Mucosa of the intussusciens.

e 2.—Mucosa of the returning portion of bowel.

e 3.—Mucosa of the entering portion of bowel.

f.—Mesentery.

Fig. 8.—Constriction of the bowel wall produced by nodules (portion of the ileum).

Fig. 9.—General view of the intestines of the Sheep.

A.—Caecum.

*B**—Extremity of "cul-de-sac" of the caecum.

C.—Terminal portion of small intestines.

D.—Floating portion of small intestines.

*D*_x, *D*_x—Portion of small intestines where intussusception usually occurs.

E.—Intussusception.

F.—Convulsions of the colon.

G.—Mesentery.

$\frac{1}{2}$ —Nodules.

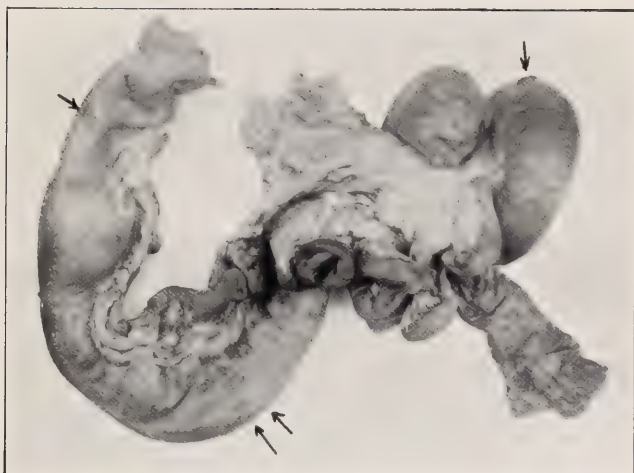


FIG. 1.



FIG. 2.

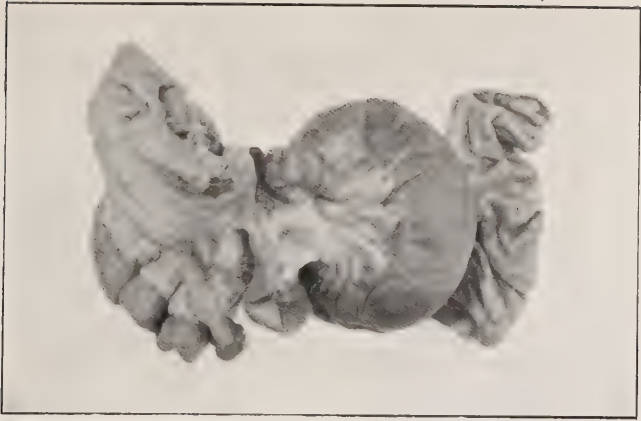


FIG. 3

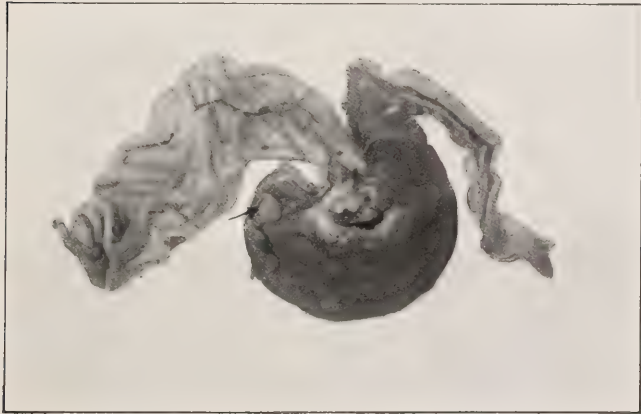


FIG. 4.



FIG. 5.

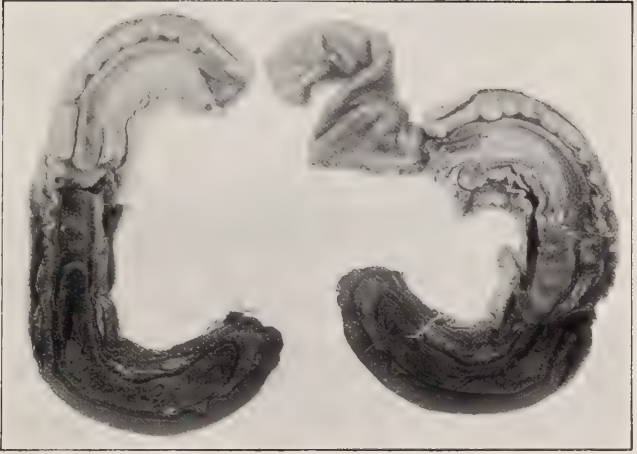


FIG. 6.

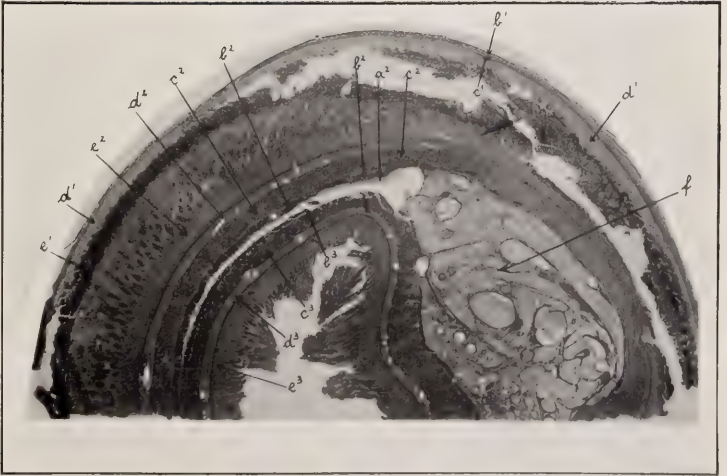


FIG. 7



FIG. 8.

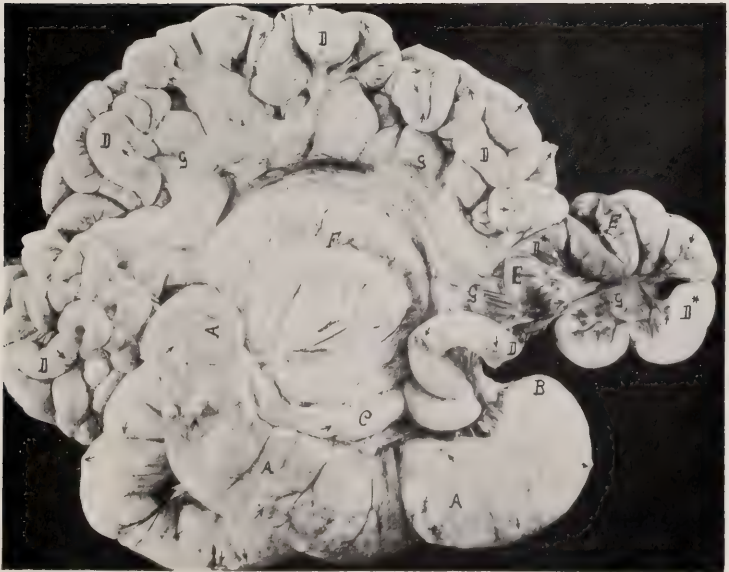


FIG. 9

The Occurrence of Dourine (Slapziekte) in South Africa

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IN February, 1914, a farmer residing in Griqualand West, Cape Province, communicated with the Veterinary Research Division, Onderstepoort, with reference to a disease known locally as "Slapziekte," which was causing serious losses not only amongst his own horses (mares), but also those of other owners farming in the immediate vicinity, and in the Barkly West Division and to the south of Griquatown, and requested that an investigation be made for the purpose of elucidating the cause. (The symptoms noted by this owner will be described later.)

Unfortunately, an officer was not available at the time, but it was suggested that one or more affected horses should be sent to Onderstepoort for investigation and observation. As the disease was occurring amongst unbroken horses, which were not kept under close observation, cases were usually observed only after locomotory symptoms had appeared, and it was then found to be impossible for the animals to travel to the rail-head, a distance of about eighty miles, and the idea had for this reason to be abandoned. However, blood from some affected animals was forwarded to the Laboratory. This was injected into horses for the purpose of ascertaining whether it would be possible to transmit the disease. Inoculation with this blood did not give any results. In July, 1914, the Principal Veterinary Officer instructed Mr. J. H. Lyons, Government Veterinary Officer, to proceed to the Herbert District and investigate the disease. Mr. Lyons visited eight farms and saw twelve cases, and observed the symptoms, which will be described later, and found (1) that the disease was principally confined to mares, although information was obtained to the effect that one stallion had died of it; (2) that not all mares on the same farm contract the disease; and (3) that cases do not occur on every farm in the same locality. He was informed that this affection had been known for about three years in the Hay and Herbert Districts. He stated that he was unable to trace infection to the stallion, and of those he had examined nothing was found wrong with the penis. Mr. Lyons forwarded blood, which was injected, but with negative results. Owing to the depletion of the staff consequent on the war, further local investigations could not be undertaken at the time. In April, 1916, the Veterinary Research Division were notified that the disease was still prevalent, and Mr. W. H. Andrews, Veterinary Research Officer, was instructed to continue the investigation, and as the result of inquiries and observations carried out "*in loco*" he arrived at the following conclusions:—

1. That the losses in the Herbert District during the previous two years must have been considerable (some hundreds).

2. There is much evidence pointing to transmission of the disease during coitus.

3. The symptoms and lesions are suggestive of rather an atypical form of Dourine, showing much resemblance to the modified form of Dourine in Canada.

4. The disease is generally said to appear during drought, and particularly during the months of September and November, but cases were seen by Mr. Andrews in May, 1916, when the veld was in fair or even good condition. According to Andrews, the unfavourable conditions of a drought in bringing out latent cases probably play the same role as that ascribed by Canadian authors to excessive work and other depressing conditions in cases of Dourine. He brought blood from the horse of which he had made a post-mortem, and on the 19th May, 1916, it was injected into a dog, six rabbits, and three rats. No positive results were obtained.

In September, 1916, the Director of Veterinary Research and Mr. Andrews, together, visited the district, when further observations were made which supported the diagnosis of Dourine, and evidence was obtained that only stallions and mares were suffering from the disease, and in the one instance of a gelding that was known to have died, it was ascertained that he had been castrated only four months previously, having thus contracted the disease as a stallion. Evidence was also obtained that at least one of the stallions had shown swellings on the prepuce and penis, but no information as to the presence of "plaques" was obtained, but it was pointed out that the mares were wild and were not kept under close observation, hence any swellings which might occur would be missed. Information was also obtained that a good many mares recover and will produce again healthy foals, whilst some are known to abort. It is however also known that, subsequent to recoveries, outbreaks can again occur. Two mares and a stallion were selected at the time of the visit and sent to Onderstepoort in September, 1916, and in January, 1917, seven more horses were forwarded. The numbers allotted to these on arrival were as follows: *Arrived September, 1916:* Horse 10679, bay mare, aged. Horse 10680, dun mare, aged. Horse 10681, bay stallion, aged. *Arrived January, 1917:* Horse 10970, roan mare, seven years old. Horse 10971, light bay mare, rising five. Horse 10972, bay mare, aged. Horse 10973, bay mare, four years old. Horse 10974, chestnut mare, three years old. Horse 10975, bay mare, six years old. Horse 10976, chestnut mare, four years. Besides the above-mentioned animals, Horse 11776, black mare, nine years old, and Horse 11782, brown mare, five years old, arrived at the Laboratory on the 3rd April, 1918, and 13th April, 1918, respectively. The animals which arrived in September, 1916, and January, 1917, were kept under observation by Mr. Andrews from the date of their arrival until the beginning of February, 1917. This officer made a number of transmission experiments and microscopically examined material collected at various times from these, as well as from the horses and small animals which were put in experiment.

In February, 1917, the writer was instructed by the Director of Veterinary Research, in the absence of Mr. Andrews, on leave, to continue the search for the causal organism and to carry out further transmission experiments. Mr. Kearney, M.R.C.V.S., Veterinary Officer of the Veterinary Research Laboratory, Nairobi, British East Africa, who

was on a visit to the Laboratory, Onderstepoort, from February, 1917, to May, 1917, rendered considerable assistance, particularly in the microscopical examination of material. During the course of the investigations, the writer made post-mortem examinations on some of the above-mentioned animals and collected material for histological examination, with the result that nerve changes which must be interpreted as those of a neuritis were found and in some cases well pronounced. Taking into consideration the satisfactory results obtained by Canadian workers from the complement fixation tests in Dourine, it was decided to adopt this method as well for confirming the diagnosis, but this could not be carried out in Onderstepoort as the causal agent had not up to then been found and a culture was not available. Serum was collected from the remaining affected animals and from those in transmission experiments, and were submitted, together with the serum of four control animals, to Professor Watson, of the Veterinary Research Laboratory, Alta, Dominion of Canada, who may be considered an authority on complement fixing tests in Dourine. The return given by the Pathologist of the Institution, in the absence of Professor Watson on military duty, was positive in the case of those horses which had a previous history of or showed symptoms of the disease. Serum from three horses which were transfused with large quantities of blood of affected horses also gave positive results. In March, 1918, the writer was instructed to proceed to Kimberley in connection with a wire received from Mr. Buck, M.R.C.V.S., Kimberley, reporting the occurrence of Dourine (Slapziekte) on two farms in the vicinity, viz., farms G and D. On arrival at farm G it was found that the affected animal had died the night previous to the day of visit, and the carcass was too decomposed for collection and examination of material. The history and symptoms furnished by the owner corresponded with those of slapziekte. At farm D a black nine-year-old mare was submitted for examination. The animal was in good condition and had been noticed affected for about five months. Symptoms usually found in the second stage of the disease, viz., locomotory, were pronounced. She was only partly broken in and difficult to handle, and as the owner kindly placed her at my disposal, it was decided to send the mare to the Laboratory for investigation and further observations. She arrived on the 3rd April, 1918, in a state of collapse, and died on the evening of the day of arrival. Prior to death, blood was collected and injected intraperitoneally to rabbits, guinea pigs, white rats, white mice, and an adult dog and pup 1408, and on the 9th April, 1918, *Trypanosoma equiperdum* was detected in a blood-stained preparation collected from pup 1408. During this visit to Kimberley District a five-year-old mare on farm V was also examined. This animal was showing the following symptoms: Copious discharge from vulva; frequent urination, particularly when trotted or made to move suddenly; the tail and hocks were wetted with the discharge; the vaginal mucous membrane was reddened; the temperature was normal; condition was poor in comparison with other animals on the farm. According to the owner, similar symptoms were observed in the case of two other mares on the farm, both of which developed locomotory symptoms and died of Slapziekte. Blood was collected from the five-year-old mare at time of visit and intraperitoneally injected immediately after into rabbits, guinea pigs, white rats, and two pups with negative results to date, viz., 30th April, 1918. A number of smears of blood and discharge

from the vulva was microscopically examined with negative results. This mare, as stated above, was sent to the Laboratory on the 13th April, 1918, for investigation and observation purposes, and at the time of writing is still under observation.

Transmission Experiments.—The transmission experiments which were made at various times, and the result of examination of material from affected horses, as well as the results of the observations and examination of material of injected animals, are given hereafter. The blood used for transmission experiments was undefibrinated and injected immediately after collection. The uterus and vagina were washed with sterile physiological salt solution and the washings utilized immediately after collection. The spinal fluid was collected immediately after death and injected immediately after collection. The microscopical examination of material was made with fresh and stained preparations.

1. *Horse 10680.*—On the 4th October, 1916, mucus of the vagina and scrapings of the ulcers were injected into four rabbits and twenty white mice; on the 6th October, 1916, 5000 c.c. of blood infused into horse 10344 and, again, a similar quantity on the 23rd November, 1916. On the 2nd January, 1917, three dogs were injected with blood. On the 27th August, 1917, the mare was killed and 50 c.c. of her spinal fluid were injected into eight rabbits, six guinea pigs, and twenty white mice. On the 15th February, 1917, the serum of this mare 10680 was utilized to inject subcutaneously a number of horses: 10 c.c., 9546, 10679, 10970, 10971; 15 c.c., 9597, 10681, 10972, 10973; 20 c.c., 9536, 10344, 10382, 10974, 10975, 10976. On the 23rd October, 1916, horse 10680 was injected with 15 c.c. serum of horse 10679. All these inoculations were made to induce local reaction, in which, according to the experience of the Canadian workers, the presence of trypanosomes might be traced. The results were negative.

2. *Horse 10681.*—On the 6th October, 1916, and again on the 23rd November, 1916, 5000 c.c. blood were infused into horse 9597. No positive results were obtained from this infusion.

3. *Horse 10679.*—On the 6th October, 1916, and again on the 23rd November, 1916, 5000 c.c. blood transfused into horse 9446. On the 13th October, 1916, some vulval discharge and scrapings from some of the ulcers present at that time were injected into four guinea-pigs and seven rabbits. On the 19th April, 1917, some fluid of what was considered to be a plaque was obtained and injected into three rats and three mice. There were no positive results from these injections. The mare apparently recovered and was still under observation at the end of 1917.

4. *Horse 10973.*—On the 5th February, 1917, 5000 c.c. of her blood were infused into horse 9597. No positive results were noted.

5. *Horse 10975.*—On the 5th February, 1917, 5000 c.c. of her blood were infused into horse 10344. On the 14th February, 1917, twenty mice were injected with serous fluid of what appeared to be a plaque. Injections with similar material were made on the 9th May, 1917, to two rabbits and six white mice; on the 24th September, 1917, to four guinea-pigs, two white rats, and six mice. On the 1st October, 1917, fresh blood was injected into four dogs. All these injections gave negative results.

6. *Horse 10971*.—Of her blood 5000 c.c. were infused into horse 9546 on the 5th February, 1917, without any positive result.

7. *Horse 10344* showed some fever reaction and on the 21st December, 1916, was bled and 20 c.c. of blood were injected subcutaneously into three dogs, 2-3 c.c. into five rabbits, and $\frac{1}{4}$ - $\frac{1}{2}$ c.c. into twenty white mice.

8. *Horse 10972*.—On the 5th February, 1917, horse 9597 received an infusion of 5000 c.c. blood, and on the 28th March, 1917, an injection of 150 c.c. blood; at the same date horse 10344 received the same quantity. On the 10th April, 1917, horse 10972 was killed and the following injections were made: with spinal fluids—intraperitoneally, two dogs and two rabbits and four white mice; intraocularly, two dogs and two rabbits; subcutaneously, two mice with fluid from the subarachnoideal space; intraperitoneally, two dogs and two rabbits, two white rats; and subcutaneously, two mice with emulsion of spinal cord; intraperitoneally, two dogs with washings of the uterus; and intraperitoneally, two white rats with washings from the vagina. No positive results were obtained. On the 25th March, 1917, washings of the vagina were injected into the vagina of horse 10976. Subsequently, at intervals, blood examinations made were negative; likewise on the 11th January, 1918, smears made from an oedematous swelling of the forearm.

9. *Horse 10970*.—Infusion from this horse to the amount of 5000 c.c. was made into horse 10970 on the 5th February, 1917. Subsequent examinations were all negative. On the 4th May, 1917, the horse was killed and the following injections were made: intraperitoneally, two dogs, two rabbits, four white rats, and two mice; intraocularly, two dogs, two rabbits; intravaginally, seven horses, viz., 9511, 9597, 9602, 9646, 10344, 10382, 10676, with fluid collected from the subarachnoideal space; intraperitoneally, two dogs, two rabbits, two white rats, and two mice with emulsion of the spinal cord. Subsequently one of the horses showed fever reactions, viz., horse 9597, on the 13th July, 1917-17th July, 1917, with a maximum of 106° F., and on the 2nd August, 1917-6th August, 1917, with a maximum of 105.4° F. The blood examinations on the date mentioned were negative and there were no results in any of the other animals injected.

10. *Horse 11776*.—On the 27th March, 1918, the animal was tapped and the blood was injected intraperitoneally into four rabbits in the dose of 10 c.c.; four guinea pigs, 5 c.c.; six white rats, 2 c.c.; six white mice, $\frac{1}{2}$ c.c.; into pup 1408 and into dog 1379, 30 c.c. On the 9th April, 1918, pup 1408 showed the presence of *Trypanosoma equiperdum* scantily in its blood; up to the 24th April, 1918, all other animals remained negative.

Experiments to transmit Dourine from affected mares to a healthy stallion—

• *Stallion 9743*, which for some time was in Onderstepoort, was used to serve affected mares, viz., on the 15th November, 1916, mare 10680; on the 26th February, 1917, mare 10976; on the 27th February, 1917, mares 10680 and 10679; on the 17th March, 1917, and 25th March, 1917, mare 10976. On the 13th March, 1917, a plaque appeared on the neck about the size of a hand. It was punctured and serum was

examined with negative results. Some of the fluid was then injected into eight white mice; no results were obtained. On the same date a red sore was noted on the penis, scrapings of it when examined were negative. On the 17th April, 1917, a swelling on the abdomen occurred; it was punctured and examined with negative results, and then, intraperitoneally, were injected six rabbits, six rats, and six mice, all of which remained healthy. On the 19th April, 1917, again, what appeared to be a plaque, was examined with negative results, and one dog injected intraperitoneally with 10 c.c. of the fluid remained healthy. On the 19th April, 1917, on the occasion of a temperature exacerbation, blood was examined with negative results, and, on the 10th May, 1917, serum of what appeared to be a plaque, also with negative results.

Serum was collected on the 20th April, 1917, and submitted to the complement test which proved negative.

Experiments to transmit Dourine by means of an affected stallion to healthy mares—

Stallion 10681 was used to serve the mares 9569 on the 2nd November, 1916 and 10382 on the 3rd November, 1916, and 9536 on the 4th November, 1916.

Results: On the 6th March, 1917, suspicion was raised about a vaginal discharge in mare 9569 and examination made which proved negative. Complement deviation tests gave negative results.

The blood of mare 10382 was examined with negative results. An injection of serum of the affected horse 10680 raised no swelling on 15th February, 1917. She aborted on the 10th April, 1917. The vaginal discharge, examined two days later, gave negative results, and injection of 30 c.c. spinal fluid into the vaginal wall on the 4th May, 1917, from horse 10970 remained without effect. She was subsequently used in a pernicious anaemia experiment and died from this disease. Of her blood were injected four dogs, six guinea-pigs, four rabbits, and all remained healthy. The complement deviation test gave negative results. Of mare 9536, blood smear examinations were made on several occasions from a suspicious discharge from the vulva, all with negative results. The complement deviation tests were negative. On the 13th November, 1916, vaginal mucus was injected into six rabbits and ten white mice with negative results.

Summary of Transmission Experiments.—In this summary, mares which had a previous history of, or showed symptoms of, the disease whilst at Onderstepoort are described as *affected horses*:—

1. Transfusion and injection of blood of affected horses into healthy horses, total 13. (Transfused horses positive to complement fixation test.) Result, negative.

2. Injection of fresh undefibrinated blood of affected horses to nine rabbits, six guinea-pigs, nine white rats, eight white mice, three adult dogs, and four pups intraperitoneally, and seven adult dogs subcutaneously; total, 46. Result: One experiment positive; remainder, negative.

3. Injection of serum of affected horses into affected horses. Results, negative; total 10.

4. Injection of serum of affected to healthy horses; total, 5. Results, negative.

5. Injection of fluid from plaques and oedematous swellings of affected horses to two rabbits, four guinea-pigs, five white rats, and thirty-five white mice; total, 46. Results, negative.

6. Injection of fluid from plaques and oedematous swellings of healthy stallion used for coitus experiment into six rabbits, six white rats, fourteen white mice, and one dog; total, 27. Results, negative.

7. Washings of uterus of affected horses to dogs; total, 2. Results, negative.

8. Vaginal washings of affected horses to small animals, white rats, intraperitoneally; total, 2. Results, negative.

9. Vaginal washings of affected horse to affected horse inter-vaginal. Results, negative.

10. Discharge from vulva and scrapings of ulcers in vulva of affected horse into seven rabbits and four guinea-pigs, subcutaneously; total, 11. Results, negative.

11. Vaginal mucus and scrapings of ulcers in vulva of affected horse into four rabbits and twenty white mice, subcutaneously; total, 24. Results, negative.

12. Vaginal mucus from affected horse in coitus transmission experiment into six rabbits and ten white mice, subcutaneously; total, 16. Results, negative.

13. *Coitus Experiments*.—Infected mare with healthy stallion; total, six. Results, negative. Infected stallion with healthy mares; total, three; results, negative.

14. Injection of spinal fluid from affected horses to healthy horses and small animals and dogs: Seven horses, intravaginal mucosa; four rabbits, eight white rats, two white mice, four adult dogs, intraperitoneally; twelve rabbits, one white rat, twenty-nine white mice, one adult dog, six guinea-pigs, subcutaneously; and four rabbits and four adult dogs, intraocularly; total, 82. Results, negative.

15. Injection of emulsion (in physiological salt solution) of spinal cord of affected horses to small animals and dogs: Four rabbits, four white rats, four white mice, four adult dogs, intraperitoneally; and one rabbit, one white mouse, and one adult dog, subcutaneously; total, 19. Results, negative.

Complement deviation test of horses which had a previous history of, or showed symptoms of, the disease whilst under observation at Onderstepoort—

Horse 10679: Showed slight paraplegia, discharge from vulva: appears to be recovering. Complement deviation test, positive. Horse 10680: Poor condition; apparently recovering. Complement deviation test, positive. Horse 10681: Appears to have recovered. Complement deviation test, positive. Horse 10970: Partial paraplegia, poor condition; appears to be recovering. Complement deviation test, positive. Horse 10971: Poor condition; apparently recovering. Complement deviation test, positive. Horse 10972: Emaciation and anaemia, constant discharge from vulva. Complement deviation test, positive. Horse 10973: Poor condition; apparently recovering.

Complement deviation test, positive. Horse 10975: Poor condition; apparently recovering. Complement deviation test, positive. Horse 10976: Intermittent discharge from vulva; apparently recovering. Complement deviation test, positive. Serum was collected from the above horses for the complement deviation test on the 20th April, 1917.

Complement Deviation Test of horses in Transmission Experiments and Control Animals—

Horse 9546: 6th October, 1916, injected 5000 c.c. blood from horse 10679; 23rd November, 1916, injected 5000 c.c. blood from horse 10679; 5th February, 1917, injected 5000 c.c. blood from horse 10970 and horse 10971; 15th February, 1917, injected subcutaneously 10 c.c. serum of horse 10680. Complement deviation test, positive.

Horse 9597: 6th October, 1916, injected 5000 c.c. blood from horse 10681; 23rd November, 1916, injected 5000 c.c. blood from horse 10681; 5th February, 1917, injected 5000 c.c. blood from horses 10972 and 10973; 15th February, 1917, injected subcutaneously 15 c.c. serum of horse 10680; 4th May, 1917, injected intra mucosa vaginalis 30 c.c. spinal fluid of horse 10970. Complement deviation test, positive.

Horse 10344: 6th October, 1916, injected 5000 c.c. blood from horse 10680; 23rd November, 1916, injected 5000 c.c. blood from horse 10680; 5th February, 1917, injected 5000 c.c. blood from horse 10975; 15th February, 1917, injected subcutaneously 20 c.c. serum of horse 10680; 4th May, 1917, injected intra mucosa vaginalis 30 c.c. spinal fluid of horse 10970. Complement deviation test, positive.

Horses 9511, 9602, 9646, 10676: Controls. Serum was collected from the above animals for the complement deviation test on the 20th April, 1917. Results, negative.

Complement Deviation Test of horses in Transmission: Coitus Experiments—

Stallion 9743 served mares which had a previous history or showed symptoms of the disease whilst at Onderstepoort. Complement deviation test, negative. Mare 9536: Served by stallion 10681, which had a previous history and showed symptoms of the disease whilst at Onderstepoort. Complement deviation test, negative. Mare 9569: Served by stallion 10681, which had a previous history and showed symptoms of the disease whilst at Onderstepoort. Complement deviation test, negative. Mare 10382: Served by stallion 10681, which had a previous history and showed symptoms of the disease whilst at Onderstepoort. Complement deviation test, negative. Serum was collected for the complement deviation test on the 20th April, 1917.

Summary of Complement Deviation Tests—

With the exception of horse 10974, which was destroyed previous to collection of serum, serum of the above-mentioned nine horses, which had a previous history of, or showed symptoms of, the disease whilst at Onderstepoort, viz., horses 10679, 10680, 10681, 10970, 10971, 10972, 10973, 10975, and 10976, was submitted to the complement deviation test, with the result that in each case the test was returned as positive. Serum collected from horses 9546, 9597, and 10344, which had been transfused with blood and injected with serum of

horses which had a previous history of, or showed symptoms of, the disease whilst at Onderstepoort, was also returned as positive, although in these three there was no clinical evidence of the disease at the time. Serum collected from mares 9569, 10382, 9536, which were served by stallion 10681, gave negative results. Stallion 10681, notwithstanding the positive complement deviation test, did not transmit Dourine, the explanation being that at the time of copulation he probably was no longer infected. Serum collected from a stallion which had been kept at Onderstepoort since September, 1915, and which had been used for serving mares 10679, 10680, and 10976, gave a negative result, hence in this one instance the three mares were not able to infect the stallion. Serum collected from four control horses, 9511, 9646, 9602, and 10676, gave negative results.

Symptoms of the Disease.

The owner who first communicated the occurrence of the disease in West Griqualand, described the symptoms as follows:—"The first indication that something is wrong is that the animal seems somewhat, perhaps very slightly, slack in the hindquarters, straddles the legs a little, and occasionally stumbles with one hind foot, which obviously causes a good deal of pain, as the animal will half stop in its walk before going on again. This condition may continue for weeks, but in several cases the slackness of the hindquarters rapidly increased with spells of apparent improvement in some cases. Often one of the hind legs seems to be more seriously affected than the other, and is moved as in a horse suffering from stringhalt. The animal falls off rapidly in condition, and those that recover remain unthrifty for months. In the acute cases the animal gradually gets worse, and seems to lose control more and more of the hind legs, which are with difficulty, and evidently pain, carried forward in walking. Eventually the conditions become so acute that the animal lies down and has difficulty in rising; finally (and generally after having drunk its fill) it falls and entirely fails to get up again at all. The slackness appears to originate in the loins, as the hindquarters before the final collapse are seen to sway as the animal walks."

Government Veterinary Surgeon Lyons reports as follows:—

"Young mares do not seem to contract the disease. There is first seen a discharge from the vagina; this is ejected in large quantities (about a teacupful at a time) whenever the animal is made to trot, or suddenly started from a standing position. The hips and tail become quite wet. The mare shows restlessness when standing, and fidgets from one hind leg to the other. As the discharge goes on the animal loses condition fairly rapidly, and becomes tucked up. About this stage she becomes lame, generally in one and then (often) in both hind legs; the lameness or weakness, however, seems to be located between the hip and the stifle joints. The gait becomes straddled, the lame leg being swung out from the medium line when the animal tries to walk. When both legs are affected the mare has the appearance as if she wanted to get her legs as far apart as possible when trying to walk forward. There is no rise of temperature, and foals sucking do not seem to contract the disease. Some people say that mares while discharging are very desirous for the

stallion, even when in foal, others say they have never seen this desire. Mares carrying a foal often show the symptoms of the disease, and they very often abort, but not always. The mucous membrane of the eye in the early stages looks quite healthy, but in cases of long standing it becomes extremely pale. The animals are generally in good condition (in fact all the horses in the district on the veld are fat) when they first show symptoms. Some farmers say, after becoming poor, if they are given a change of feed, or put on lucerne, they pick up for a time, but improvement is not lasting."

The status presens and observations recorded in the case of the ten animals which were sent to Onderstepoort for experimental investigations are given below:—Horse 10679: Bay mare, aged; blaze, sock near hind. Date of arrival at Onderstepoort, 29th September, 1916. Status presens, 4th October, 1916. General appearance poor; hard swelling on left poll; conjunctiva yellow-red colour. Gait, short steps behind, stiff, and turns badly; dropping slightly on near hind leg. Mucosa of vulva slightly injected; no ulceration. Vagina mucosa few injected streaks; no discharge.

Observations.—On 13th October, 1916: Labia vulvae oedematous. Slight discharge from vulva, and ulcers on labia and clitoris. 24th October, 1916: Still discharge when trotted. 3rd November, 1916: Ulcers in vulva almost disappeared. 29th November, 1916: Losing condition; exercised daily. Plaques seen on 30th March, 1917, and 19th April, 1917. Discharge from vulva up to 8th May, 1917. 23rd February, 1917: A raised, irregular patch on skin between 3rd and 4th ribs. 23rd August, 1917: Condition very poor; stiff gait behind. 29th September, 1917: Again slight, clear discharge from vulva. Since the 13th October, 1916, to 29th January, 1918, date under observation, there has been a discharge of fluid from the vulva (usually clear, watery-like or slightly viscid). *Complement Deviation Test*: Positive. (Serum collected for C.D. Test 20th April, 1917.)

Horse 10680: Dun horse, aged, black legs. Date of arrival at Onderstepoort, 29th September, 1916. Status presens, 4th October, 1916. General condition very poor; tucked up; eyelids much swollen and mucopurulent discharge; conjunctiva injected; nasal membrane rather injected and very slight nasal discharge; gait, slightly lame in near hind, horse turns badly; on vulva externally dried yellowish brown discharge, and irregular shallow ulcer on edge of labium; a similar one along the summit of the clitoris; vulva mucous membrane injected in patches; vagina mucosa appears rather oedematous.

Observations—27th October, 1916: Slight muco-purulent discharge from vulva, and ulcers. 3rd November, 1916: Great improvement in health; ulcers in vulva healed out; ecchymoses in vagina. 23rd November, 1916: Labia swollen and oedematous; no discharge. 29th December, 1916: Discharge both eyes. 28th January, 1917: Vulva slightly oedematous and slight discharge. 24th April, 1917: Mucous deposit on vulva; discharge from eyes still remains. 10th May, 1917: Vulva appears normal, slightly pale. 16th July, 1917: Poorer in condition; vulva slightly oedematous. 23rd August, 1917: Very poor; mucous discharge both eyes still present. Animal down; unable to rise, and was *killed* on the 27th August, 1917.

Pathological anatomical diagnosis.—Anaemia and emaciation; slight ascites; slight hydropericardium; calcified lesions in lungs, slight liver atrophy, slight enteritis. Histological examination of heart muscle and nerve tissues—nerve sciatic; neuritis; heart muscle: normal. Complement deviation test: positive. Serum collected for C.D. test 20th April, 1917.

Horse 10681.—Bay stallion, aged; white pastern near hind. Date of arrival at Onderstepoort, 29th September, 1916. Status presens, 4th October, 1916; condition rather poor; conjunctiva slightly injected; gait rather stiff, with short step behind, but not lame; crouching appearance; penis shows no obvious abnormality.

Observations.—13th October, 1916: Oedematous swelling in scrotum. 3rd December, 1916: Scrotum normal; losing condition. 12th January, 1917: Scrotum swollen. 21st February, 1917: Oedema size half-crown on ventral aspect of scrotum. 28th February, 1917: Swelling gone; purulent discharge in nostrils. 6th March, 1917: plaque size five-shilling piece, dependent portion of scrotum. 30th March, 1917: Scrotal oedema disappeared, also discharge from nostrils. Horse improved afterwards in condition and showed no abnormality until 16th July, 1917, when the scrotum became again slightly oedematous. Still alive. Complement deviation test, positive. Serum collected for C.D. test 20th April, 1917.

Horse 10970.—Red roan mare, 7 years; "G" on left thigh. Date of arrival at Onderstepoort, 20th January, 1917. History: Contracted "slapziekte" about two years ago; improved and foaled, and showed disease about six months ago for second time. Status presens, 26th January, 1917. Condition very poor; conjunctiva slightly ecchymosed; small abscess in right temporal region; gait at the walk gives a "slack in the loins" appearance; at the trot turns badly, and appears to drop somewhat on the off hind leg; near leg much thinner in thigh than right. Mucosa of vulva and vagina appears slightly oedematous and hyperaemic, engorged veins standing out in places; one ecchymosis about 3 by 2 cm. Right mammary gland enlarged.

Observations.—20th February, 1917: Slight blood-tinged discharge from vulva. 22nd February, 1917: Mucosa of vulva slight yellowish tinge. 23rd February, 1917: Weak, straddling gait. 28th February, 1917: Has been down, very weak; drags hind legs; mucous discharge from eyes. 8th March, 1917: Wide, staggering gait behind; fresh decubitus near hip; vulva hyperaemic. 14th March, 1917: Decubitus both hocks; gait straddling. 23rd March, 1917: Vulva normal; no change otherwise. 11th April, 1917: Oedematous and hyperaemic patch size of walnut on vulva. Animal was killed on the 4th May, 1917, and material collected for histological examination and transmission experiments.

Pathological anatomical diagnosis.—Anaemia; liver atrophy. Calcified nodules in liver. Histological examination of muscles and nerve tissue—muscles: normal; nerve sciatic: neuritis. Complement deviation test, 29th June, 1917: positive. Serum collected for C.D. test 20th April, 1917.

Horse 10971.—Light bay mare, 4 rising 5; "G" left thigh. Date of arrival at Onderstepoort, 20th January, 1917. History: Has

never foaled; showed disease about four months ago. Status presens, 26th January, 1917. Condition rather poor; conjunctiva very slightly ecchymosed; not lame; vulva and vagina show nothing abnormal to the eye.

Observations.—19th February, 1917: Appears to be losing condition. 23rd February, 1917: Slight lameness, off hind. 14th March, 1917: Gait normal. 16th July, 1917: Condition improved; gait normal. Up to 10th October, 1917, no discharge noted. Up to 31st January, 1918, remained in good condition, on which date it was put in a horse-sickness experiment and died on the 12th February, 1918, of horse-sickness.

Pathological anatomical diagnosis.—That of horse-sickness, namely, slight hydrothorax, hydropericardium, petechiae in epicardium, extravasations in endocardium, gelatinous infiltration in subcutaneous tissues, hyperaemia of kidneys, fatty degeneration of liver, tumor splenis, hyperaemia of stomach and intestines. Complement deviation test, 29th June, 1917: Positive. Serum collected for C.D. test 20th April, 1917.

Horse 10972.—Bay mare, 10 years; star; "C" on right thigh. Date of arrival at Onderstepoort, 20th January, 1917. History: Has just contracted the disease. Status presens, 26th January, 1917. Condition very poor; conjunctiva pale, with a small ecchymosis; lame behind, dropping on the off leg; vulva and vagina appear normal.

Observations.—15th February, 1917: Labia slightly oedematous. 21st February, 1917: Slight muco-purulent discharge from vulva; losing condition. 28th February, 1917: Weak, poor state; drags hind legs. 4th March, 1917: Still muco-viscid discharge from vulva; hind legs swollen, much oedema. 6th March, 1917: Aborted (foetus about one month). 8th March, 1917: All legs swollen; condition improves. 14th March, 1917: Little or no swelling on legs; ecchymosis on conjunctiva. 15th March, 1917: Small oedematous patch on right labium. 23rd March, 1917: No discharge from vulva; all legs swollen again; staggering gait; condition poor; pulse slow and weak. 25th March, 1917: Discharge from vulva and vagina. 1st April, 1917: Few small haemorrhagic points on vulva; discharge semi-clear. 3rd April, 1917: Ecchymoses on vulva and frothy deposit on labia; swelling hind legs increasing. 8th April, 1917: Profuse discharge from vulva. Animal was killed on the 10th April, 1917, and material collected for histological examination and transmission experiment.

Pathological anatomical diagnosis.—Anaemia; atrophy of liver, spleen, and kidneys; cystic kidneys; some calcareous lesions in lungs. Histological examination of muscles and nerve tissue—muscles: normal; nerve sciatic: neuritis. Complement deviation test: positive. Serum collected for C.D. test 10th April, 1917.

Horse 10973.—Bay mare, 4 years; small star; sock near fore and stockings both hind legs; "CF" right leg. Date of arrival at Onderstepoort, 20th January, 1917. History: contracted disease about two months ago. Status presens, 26th January, 1917. Condition rather poor; conjunctiva pale; slightly lame behind, dropping on the off leg; no discharge from vulva; a few petechiae along the

edge of the labium just to inner side of junction of skin and mucous membrane.

Observations.—7th February, 1917: Labia appears very slightly oedematous. 21st December, 1917: Quittor caused by an injury causing much lameness. 22nd February, 1917: Slight discharge from vulva and faint swelling of labia. 14th March, 1917: A few scars on clitoris; still lame near hind; no discharge from vulva. 30th May, 1917: Thin grey discharge from vulva, and clitoris slightly oedematous. 16th July, 1917: Again normal. 2nd October, 1917: Getting in very poor condition. Since developing the quittor on the near hind foot as the result of an injury on the 10th February, 1917, there has been little or no improvement in condition. The horse was killed on 8th January, 1918.

Pathological anatomical diagnosis.—Anaemia. Atrophy of hip muscles near hind leg; necrosis of os pedis and surrounding tissue near hind; fistula extending into articulation of 2nd and 3rd phalanx; parasitic infection of stomach and intestines; bone marrow of humerus and femur haemorrhagic and gelatinous. Histological examination of muscles, nerves, tissues, etc.: muscular tissue normal (sarcosporidia rare). Sciatic nerve: neuritis. Complement deviation test, 29th June, 1917: Positive. Serum collected for C.D. test 20th April, 1917.

Horse 10974.—Chestnut mare, 3 years; "GC" on left leg. Date of arrival at Onderstepoort, 20th January, 1917. History: Has never foaled; showed disease about two months, and has just started to discharge matter from womb. Status presens, 26th January, 1917. Condition poor; decubitus left elbow region; muco-purulent discharge from right nostril. Gait: goes very wide behind, dropping much on near hind. Vulva no discharge, mucosa very slight; hyperaemic; one small white patch one inch to right side of vulva.

Observations.—4th February, 1917: Slight discharge from vulva. 7th February, 1917: No discharge, but labia slightly swollen. 15th February, 1917: Vaginal mucosa slightly congested. 20th February, 1917: Mucosa of vulva slightly hyperaemic and slight ulceration of clitoris. 22nd February, 1917: Mucosa of vulva still hyperaemic. 26th February, 1917: Vulva appears normal; very poor condition. 27th February, 1917: Down, unable to rise, and very weak; much decubitus. Animal was killed on the 27th February and material collected for histological examination and transmission experiments.

Pathological anatomical diagnosis.—Anaemia. Atrophy of liver and spleen. Histological examination of nerve tissue, sciatic nerve: neuritis. Complement deviation test: serum not tested. Animal was destroyed before serum was collected from the other animals for C.D. test.

Horse 10975.—Bay mare, 6-7 years; white spots on back. Date of arrival at Onderstepoort, 20th January, 1917. History: Has had three foals; since about eighteen months ago got better; contracted disease six months ago for second time. Status presens, 26th January, 1917; condition rather poor; not lame. Mucosa of vulva and vagina slightly oedematous, and very slightly hyperaemic; no discharge.

Observations.—16th February, 1917: two plaques and oedema in sternal region. 21st February, 1917: Plaques disappeared; oedema unchanged. 28th February, 1917: Condition of animal improving. 8th March, 1917: Slight oedema under stomach; vulva moist. 23rd March, 1917: Muco-purulent discharge of eyes. 1st April, 1917: Muco-purulent discharge near eye; slightly lame near hind. 8th May, 1917: Large plaque on right side of body. 18th June, 1917: Marked muco-purulent discharge right nostril. 24th September, 1917: Plaque (12 by 3) under chest; condition poor. 2nd October, 1917: Respirations abnormal (40); temperature 103° F.; pulse 68. 4th October, 1917: Plaques gone; vulva pale; tucked up. 2nd October, 1917, to 9th October, 1917: Temperature irregular, maximum 104° F. There has been a more or less persistent muco-purulent discharge from both eyes since the 23rd March, 1917. 6th October, 1917: Respirations 36; pulse 56; temperature normal. 13th November, 1917: Very poor in condition, oedematous swelling under chest. 19th January, 1918: Developed streptococcus equi infection. Still alive. Complement deviation test, 29th June, 1917: Positive. Serum collected for C.D. test 20th April, 1917.

Horse 10976.—Light chestnut mare, 4 years; socks both feet and near hind; "J.C." on left leg. Date of arrival at Onderstepoort, 20th January, 1917. History: Has had one foal; showed disease about eight months ago, and appears to be recovering. Status presens, 26th January, 1917. Condition fair; conjunctiva pale; not lame; drops very slightly on the off hind at the trot.

Observations.—19th February, 1917: Muco-purulent discharge from vulva: horsing. 28th February, 1917: Still horsing; near fore and off hind legs swollen. 10th March, 1917: plaque 2 by 2½ cm. on left thorax; slight watery discharge from vulva up to 11th June, 1917. 18th June, 1917: Swelling right hind leg and slight stiffness in gait. 12th July, 1917: A hard abscess on right chest wall opened. 10th October, 1917: Off hind leg still swollen; vulva normal. 11th January, 1918: Oedematous swelling under chest. Complement deviation test, 29th June, 1917: Positive. Serum collected for C.D. test 20th April, 1917.

Horse 11776.—Black mare, age 9 years. Date of arrival at Onderstepoort, 3rd April, 1918, from Kimberley. History: Contracted the disease about five months ago. Symptoms first noticed were a discharge from vulva; frequent sexual desire and discharge from vulva. Later, lameness in hindquarters. Status presens, 27th March, 1918. Condition good; when standing, weight not supported on one hind leg; knuckling over of fetlock of leg on which weight was unsupported; when moved, straddling and swaying gait of hindquarters and frequent knuckling over of hind fetlocks and dropping of hindquarters. Animal thrown for examination; nothing abnormal detected on examination of vulva and vagina. No plaques or oedematous swellings on body. Animal showed great distress after being thrown, and had considerable difficulty in rising. On arrival at the Laboratory on the 3rd April, 1918, it was in a state of collapse, muscular tremors were marked; respirations were markedly abnormal. lower lip pendulous and paralysed. Decubitus marked. Died evening of day of arrival.

Pathological anatomical diagnosis.—Broncho-pneumonia. Histological examination of nerve tissue, sciatic nerve: neuritis.

Analysis of Symptoms.

In Slapziekte no definite observations have been made as to the period which elapses between the infective coitus and the first appearance of symptoms. As already stated, animals running on a farm are not kept under close observation, and the disease has progressed somewhat before symptoms are noticed. According to text-books, the incubative period varies from five to six days to a month, sometimes longer. Usually the first indication in mares that something is amiss is a discharge from the vulva; this is most pronounced when the animal is made to move suddenly or when trotted. The discharge, which usually includes some urine, wettens the tail and legs as far as the hocks. When the mare is kept stabled there may be less discharge, and this becomes dried on the above-mentioned parts. The vulva is sometimes swollen, usually hyperaemic. Nodules and ulcers may occur on the mucosa of the vulva, vagina, and clitoris. In some cases an increased sexual desire and frequent urination has been noted. According to textbooks the ulcers may increase in size and become covered with necrotic substance, and are surrounded with raised walls, which heal slowly and leave scars behind, and the swelling may extend from the vulva to the region of the udder, inside of the legs, and abdomen. Such cases did not come under our notice, although a discharge from the vulva persisted for months, even in the case of animals which were apparently recovering.

The description given in the text-books of symptoms which occur in the stallion, such as swelling of the sheath, swelling of the penis, and nodules or ulcers on the glans penis, or body of the penis, as well as increased sexual desire and erections and frequent micturition, were not observed in the stallion 10681. A more or less persistent oedematous swelling was noted on the dependent portion of the scrotum of this stallion, but examination of fluid from this gave negative results, and it is doubtful whether this swelling was connected with Dourine.

The second stage manifests itself in disorders of the nervous system, such as lameness in one or both hind legs, varying from a stiffness in gait to marked lameness; there may be a straddling or swaying gait. There may be restlessness of the hind legs, the weight being supported first on the one leg and then on the other; the fetlock of the leg not supporting weight is flexed; when made to move the toes of the hind feet may be dragged along the ground. In some of the cases under observation at Onderstepoort, these locomotory symptoms disappeared, and the animal appeared to recover and improve in condition. On the other hand, they also remained. Wasting of the muscles of the hindquarters ensued, and the animal had frequently to be assisted to rise, and finally became so weak and emaciated that it was destroyed, paleness of the visible mucous membrane being noted in the poor-conditioned animals. Besides the above-mentioned symptoms, a chronic conjunctivitis was observed in two cases. The occurrence of "plaques," according to the text-books, is pathognomonic for Dourine. In some instances the occurrence of swellings was noted, but in no case was the parasite of Dourine

detected in smears made from the contents of these, and the transmission experiments with fluid from the swellings to small animals also gave negative results. Similar swellings were noted in the case of healthy horses, which were no doubt due to horse flies (*Tabanidae*). Exacerbations of temperature were sometimes noted in the horses under observation; blood examination, however, gave negative results, and it is probable that they were due to various causes.

Pathological Anatomy.—Post-mortem examinations were made on the following horses that were killed owing to poor condition, viz., 10680, 10970, 10972, 10973, 10974, but no typical lesions were observed. Poor condition and anaemia was found in each case. Icterus, slight hydrothorax, and ascites were usually found. Chronic atrophy of the liver or spleen was noted. In one instance cysts containing urine were observed in the kidneys, calcified nodules, due to parasitic metastases were found in some instances in the liver or lungs. Horse 10971, which had apparently recovered, was put in a horse-sickness experiment and died of horse-sickness. The post-mortem showed lesions characteristic of horse-sickness. Horse 11776, which died shortly after arrival, showed a diffuse broncho-pneumonia, which apparently originated from a previous localized broncho-pneumonia due to mechanical causes. At the time of post-mortem, portions of sciatic nerve, heart, and skeleton muscles were collected from horses 10680, 10970, 10972, 10973, 10974, 11776. Sections were cut on the freezing microtome, after fixation in 10 per cent. formalin solution, and examined after staining with haematoxylin, haematoxylin eosin, and haematoxylin sudan, or scharlach, with the result that changes were observed in the sciatic nerves, which must be interpreted as a neuritis. These comprised the following:—A cellular infiltration, an increase of the nuclei of the endoneurium, atrophy of some fibres, and, in one instance, horse 10970, a fatty degeneration of some of the nerve fibres. The epineurium showed a round cell infiltration, in some places well marked. The muscular tissue showed no degenerative changes. A slight invasion of sarcosporidia was noted in some muscle sections. The results of examination are as follows:—Microscopical examination of sections.—Horse 10680—Sciatic nerve: Round cell infiltration; some atrophy of nerve fibres. Horse 10974—Sciatic nerve: Round cell infiltration; some atrophy of nerve fibres. Horse 11776—Sciatic nerve: Marked round cell infiltration. Horse 10972—Muscles: (1) *Gluteus* (Haem. Eosin): Striation distinct; no sarcosporidia seen. (Sudan): No fatty degeneration. (2) *Quad. Ext. Cruris* (Haem. Eosin): Striation distinct; no sarcosporidia seen. (Sudan): No fatty degeneration. (3) *Supra spinatus* (Haem. Eosin): Striation distinct; no sarcosporidia seen. (Sudan): No fatty degeneration. (4) *Triceps Ext. brachii* (Haem. Eosin): Striation distinct; no sarcosporidia seen. (Sudan): No fatty degeneration. (5) *Longissimus Dorsi* (H. em. Eosin): Striation distinct; no sarcosporidia seen. (Sudan): No fatty degeneration. (6) *Psoas muscles* (Haem. Eosin): Striation distinct; no sarcosporidia seen. (Sudan): No fatty degeneration. (7) *Masseter* (Haem. Eosin): Striation distinct; no sarcosporidia seen. (Sudan): No fatty degeneration. (8) *Splenius* (Haem. Eosin): Striation distinct; no sarcosporidia seen. (Sudan): No fatty degeneration. (9) Sciatic nerve: Round cell infiltration.

Horse 10970.—Muscles: (1) Gluteus (Haem. Eosin): No sarcosporidia seen; nuclei of muscle fibres well stained; striation distinct. In one part of the section some intramuscular haemorrhage. (Sudan): No fatty degeneration. (2) Quad. Ext. Cruris (Haem. Eosin): Striation well marked; no sarcosporidia seen. (Sudan): No fatty degeneration. (3) Supra spinatus (Haem. Eosin): Striation distinct; no sarcosporidia seen. (Sudan): No fatty degeneration. (4) Triceps Ext. brachii (Haem. Eosin): Striation distinct; no sarcosporidia seen. (Sudan): No fatty degeneration. (5) Longissimus Dorsi (Haem. Eosin): Striation distinct; no sarcosporidia seen. (Sudan): No fatty degeneration. (6) Psoas Muscles (Haem. Eosin): Striation distinct in majority of fibres; no sarcosporidia seen. (Sudan): No fatty degeneration. (7) Masseter Muscle (Haem. Eosin): Striation distinct; some located haemorrhages; no sarcosporidia seen. (Sudan): No fatty degeneration. (8) Splenius Muscle (Haem. Eosin): Striation distinct; no sarcosporidia. (Sudan): No fatty degeneration. (9) Sciatic nerve: Round cell infiltration; fatty degeneration of some nerve fibres.

Horse 10973.—Muscles: (1) Gluteus (Haem. Eosin): Sarcosporidia rare; striations distinct. (Sudan): No fatty degeneration. (2) Quad. Ext. Cruris (Haem. Eosin): Striations distinct. (Sudan): No fatty degeneration. (3) Supra Spinatus (Haem. Eosin): Striations marked. (Sudan): No fatty degeneration. (4) Triceps Ext. brachii (Haem. Eosin): Striations distinct. (Sudan): No fatty degeneration. (5) Longissimus Dorsi (Haem. Eosin): Striations distinct. (Sudan): No fatty degeneration. (6) Psoas Muscles (Haem. Eosin): Striations distinct; sarcosporidia rare. (Sudan): No fatty degeneration. (7) Masseter Muscle (Haem. Eosin): Striation distinct; some haemorrhage in the intramuscular connective tissue; sarcosporidia rare. (Sudan): No fatty degeneration. (8) Splenius Muscle (Haem. Eosin): Striations distinct; sarcosporidia rare. (Sudan): No fatty degeneration. (9) Sciatic nerve (Haem. Eosin): Round cell infiltration; some atrophy of nerve fibres.

Conclusions.

(1) The Slapziekte of horses which occurs in Griqualand West and adjacent areas is identical with Dourine of Europe and other countries (Canada, United States of America).

(2) The diagnosis is based on the Epizootology, Symptomatology, Pathological Anatomy, Serum Tests [complement fixation (deviation)], and the demonstration of the causal agent (*Trypanosoma equiperdum*).

(3) The difficulty in demonstrating the causal parasite corresponds with the experience of Continental, Canadian, and other workers.

(4) Dourine has recently been observed in South-West Africa, and from the history probably existed there prior to the commencement of the German South-West campaign.

(5) It has not as yet been definitely ascertained whether it was introduced into the Union of South Africa from that territory or from oversea countries in which it is known to exist, e.g. Canada, North and South America, Russia, or from elsewhere, from where in the past importations of horses were made into South Africa.

Anthrax in South Africa.

BY

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By reason of its greatly increased prevalence and spread within the Union, Anthrax has latterly come to be one of the most serious—if not the most serious—of stock diseases in this country. On this account it has been deemed advisable to offer a review of our position in regard to its history in the country, and to its present significance.

In the following article, written not only for the veterinarian, but also for the stockowner, there have, therefore, been collected and set forth the various observations which have been made concerning the disease as it occurs under South African conditions. These observations have been gathered from all available sources, such as reports and private communications of different veterinary administrative officials, private communications of colleagues of the staff of the Veterinary Research Division, and correspondence which has passed between these Laboratories and owners of stock throughout the country. In recording them the plan adopted has been to embody them as required in the different sections into which the subject-matter of the paper has been divided. For the sake of convenience and treatment, these sections are arranged as follows:—(1) Historical; (2) Prevalence of the Disease; (3) Animals Affected; (4) Spread of Infection; (5) Diagnosis; (6) Control of the Disease: (a) Regulations for Control and Suppression, (b) Preventive Vaccination. In the treatment of the whole subject-material attention has been almost entirely confined to the consideration of the disease as it occurs and is met with under the conditions of this country. This, while evident throughout, will perhaps be most apparent from Section 4, in which the source and spread of infection are dealt with, since in this section are described certain conditions favouring the dissemination of the disease, which have particular importance as far as South Africa is concerned, but have not the same importance in other countries. Where, however, observations made in any other country appear to have a bearing on the disease as it occurs within South Africa, these have been either cited by reference or else included in the text. It is hoped that the prolixity of explanation in some sections will not prove intolerable to the veterinarian nor the citation of literature wearisome to the general reader. The aim of the article renders both inevitable.

1. HISTORICAL.

For obvious reasons it is impossible to give any definite account of the very early history of Anthrax in South Africa, but that it has existed here for very many years would seem very probable. Earlier European inhabitants and travellers through the country may have recognized a disease with appearances similar to a condition passing under the name of Anthrax or its equivalent in the countries from whence they came, but if so the writer is unaware of any record where a definite description of such a disease is given.

It is very probable that it is Anthrax to which Livingstone ⁽¹⁾ refers in his "First Expedition to Africa," describing his travels in South Africa between the years 1849 to 1856. In one chapter in this book he deals with the influence which the existence of "tse-tse fly disease" and horse-sickness—which he terms peri-pneumonia—probably had in preventing the Hottentots, coming from the north-east, from introducing the horse into South Africa, and, immediately following upon this, remarks:—

"When the flesh of animals that have died of peri-pneumonia is eaten it causes a malignant carbuncle; and when this appears over any important organ, it proves rapidly fatal. It is more especially dangerous over the pit of the stomach. The effects of the poison have been experienced by missionaries who had partaken of food not visibly affected by the disease. Many of the Bakwains who persisted in devouring the flesh of animals which had perished from this distemper died in consequence. The virus is destroyed neither by boiling nor roasting."

In this statement it is the reference to the development of a so-called malignant carbuncle in certain individuals under the conditions referred to which suggests the possibility of the animals from which the flesh was derived having died from Anthrax. Many years later than this, reference to Anthrax by Wiltshire is to be found in a Government Notice dated 1877, and incorporated in the report of a commission appointed to inquire into the existence of Redwater among cattle ⁽²⁾. That, however, this writer at that time regarded "Redwater" as identical with Anthrax is evident from a statement to the effect that "the disease in question therefore is a charbonous—or anthrax—fever." In another Government Notice ⁽³⁾ to be found in the same report, and dated 1882, the same writer again refers to Anthrax in describing "the different forms of the disease in Natal," but here confusion not only with Redwater, but also with many other diseases, is to be noted from the following extract:—

"As I have said before, Anthrax manifests itself in many different forms, and in South Africa those most generally known are Horse-sickness, Redwater, Spon-sickness, and certain cases amongst buck and other game, which I have never seen, but which have proved their nature by infecting human beings who have handled the carcasses. Besides these, however, there is the fever which prevails amongst horses,* especially of late years, and which I have lately seen in cattle also. Gloss Anthrax (or carbuncle of the tongue) prevailed also at one time, and I have a strong impression that many of the cases called "Gall-sickness" and "Bush-sickness" are really modifications of the disease. Amongst sheep and goats I have not recognized it so far, although I feel certain it exists, and may be credited with a share of the losses suffered annually."

About the same time, in 1881, Lambert, ⁽⁵⁾ making investigations into the cause of Horse-sickness in Natal, came to the conclusion that this disease was identical with Anthrax—a mistake the same as that made as late as 1895 by Sander ⁽⁶⁾ when investigating Horse-sickness in German South-West Africa.

In his yearly report for 1882, however, Hutcheon describes the finding of *Bacillus anthracis* microscopically in the blood and organs of sheep dying under conditions and with symptoms suggesting

* The fever referred to would appear to be that later known as "Biliary-fever," for Hutcheon ⁽⁴⁾ in 1883 writes: "I will endeavour to describe one or two diseases of the horses which I have seen mistaken for bots. The first and principal one amongst these I propose to call Biliary-fever. The disease to which I refer is very closely described by Mr. Wiltshire, Colonial Veterinary Surgeon for Natal; in his report on Anthrax in the *Natal Almanac* for 1883 he terms it Anthrax Fever."

Anthrax. He also there differentiates between Anthrax and other diseases, such as Sponziecte (Blackquarter), Redwater, Biliary-fever, Horse-sickness, and Heart-water; and it may be of interest to note that the first mention of Pasteurian vaccination in South Africa is here found, reference being made in reporting results obtained in inoculating sheep.

2. PREVALENCE OF THE DISEASE.

In regard to the relative prevalence of Anthrax within the Union during recent as compared with former years, the evidence which we have to consider is that contained in the official records relating to the existence of the disease during different years in the various Provinces. In reviewing this evidence it is a matter of convenience to consider it in regard to two periods, one prior to the year 1904 and the other subsequent to that time, since it is only from this year onwards that statistics concerning annual outbreaks are obtainable. From 1882 up to 1904 frequent mention of the disease as far as Cape Colony is concerned is to be found in the annual reports of Hutcheon, then Colonial Veterinary Surgeon of the Cape of Good Hope. In one of these reports reference is also made to the disease in Natal, where in discussing some of Wiltshire's findings Hutcheon, so early as 1883, states:—"Anthrax is more prevalent in Natal, and assumes a more virulent character than in most parts of this country." From the statistics contained in these reports it would appear that within the period under discussion Anthrax was recognized as being existent in many of the districts of Cape Colony. Year by year there would seem to have been an increase in the number of cases or outbreaks which occurred, since in his report for the year 1902 he remarks, speaking of Anthrax along with Quarter-evil (Sponziecte) and Redwater:—

"These diseases have been largely on the increase during the last number of years, and a large number of valuable animals have been lost which would have been saved if the methods of preventive inoculation which are now well known had been adopted."

That, however, in certain special districts the prevalence and spread of the disease appears to have been unusually marked is apparent from the following extracts, which, being of particular interest, are here given at length:—

Hutcheon: Report of the C.V.S., Cape of Good Hope, for the year 1896. *Anthrax or Meltziecte*.—"This disease has been unusually prevalent in Bechuanaland and Griqualand West, more particularly amongst horses, but numbers of cattle have also died from it. It will be observed that Mr. Soga mentions in his report that Anthrax is a well-known disease among the Baralongs, and has existed in the country for years. The cattle introduced from the different States and Colonies become virulently affected, the indigenous cattle not so severely, these latter becoming in course of time naturally inoculated. Be that as it may, there is no doubt that the disease has increased in intensity very much during late years, and is annually extending its area of infection. As soon as the disease made its appearance this year, I requested the Under-Secretary for Agriculture to cable for Anthrax vaccine from the Pasteur Laboratory at Paris. About 8000 doses were obtained, but we would have required three times the quantity to have met all the demands that came in for it, so widespread was the disease throughout Griqualand West, Bechuanaland, and the British Protectorate. It will be necessary before next spring to arrange for a regular supply of the vaccine as I anticipate an increasing demand for it. There can be little doubt that this disease has been largely spread in the first instance by the natives in these territories never taking the trouble to bury the carcasses of animals that have died of it, and I regret to report that

many of the European farmers are little better in that respect, for even during the prevalence of the disease this season I have passed over farms where apparently no effort was made to bury the carcasses or to isolate the infected from the healthy animals. After the disease appears amongst horses it is very evident that the horse-flies, so abundant in these territories, are one of the principal means of spreading it. This makes it all the more necessary that the sick animals should be removed from the healthy at once, and it should be made compulsory, under a heavy fine, for owners to bury the carcass of every animal dying from the disease."

Hutcheon: Report of the C.V.S., Cape of Good Hope, for the year 1898, *Anthrax or Meltziekte*.—"This disease is unfortunately becoming more and more prevalent every year in the territory of Griqualand West and in British Bechuanaland. This is mainly due to the culpable negligence of the majority of occupiers of farms in these parts, in not burning or burying properly the carcasses of animals which die of that disease. Those who are owners of farms ought in their own interest to attend to this matter, as every spot where a carcass has lain becomes an additional centre of infection, and these are becoming multiplied every year. There are a large number of farms in these territories now, on which the disease has become so prevalent, that, unless inoculation is resorted to, more than half of the stock frequently die."

Dixon: Report of the C.V.S. of the Cape of Good Hope for the year 1903, *Anthrax and Quarter-evil*.—"These two diseases have been unusually prevalent all over the East London, Kingwilliamstown, and Komgha Districts since I have been stationed here. Anthrax, I found, was chiefly affecting oxen riding transport, and on several occasions I have been called to examine oxen that have fallen dead in the streets of East London from this disease. I am afraid the outspans along the main Transkeian roads are badly infected with the Anthrax organism, for numbers of oxen have died from this disease at three different outspans, and no precautions have been taken to properly bury the carcasses."

These abstracts provide some conception of the conditions present in regard to the portions of the country for which evidence is available before 1904. Some idea of conditions existing since that time may be gathered from the following prevalence table which for the most part explains itself:—

TABLE compiled from the official Report of the Principal Veterinary Officers of the present Provinces of the Union prior to date of Union (1910), and from the Reports of the Principal Veterinary Officer, Union, subsequent to that date, to show the number of Anthrax outbreaks recorded annually in the different Provinces within the period 1903-1904 to 1916-1917.

	Trans- vaal.	Orange Free State.		Cape Colony.	Trans- keian Terri- tories.	Natal.
Year ended 30th June—			Year ended 31st December—			
1904.....	3	2	1904.....	28	—	—
1905.....	11	4	1905.....	45	—	—
1906.....	10	4	1906.....	66	—	16*
1907.....	9	3	1907.....	63	—	—
1908.....	13	3	1908.....	39	—	—
1909.....	17	1	1909.....	82	—	—
1910.....	39	1	1910.....	86	24†	—

* Taken from the Report of the Principal Veterinary Officer, Natal, for year ended 30th June, 1906, the only report from this Province made prior to Union obtainable.

† From 1910 onwards the outbreaks occurring annually in the Transkeian Territories are stated separately from those occurring in Cape Colony. Previous to that year the reports for both of these present separate veterinary areas or circles appear under the returns for Cape Colony.

In viewing the figures immediately below an overlapping of those furnished for the Transvaal and Orange Free State for the year 1910 will be noted. This is due to the fact that during the first two years of Union the official year commenced on the 1st January and terminated on the 31st December. The third row of figures shows the number of outbreaks within a period of fifteen months, from the 1st April, 1913, to 31st March, 1914, due to readjustment of the year which from 1914 commences on the 1st April and terminates on the 31st March.

	Trans- vaal.	Orange Free State.	Cape Colony.	Transkeian Territories.	Natal.
Year ended 31st December—					
1910.....	57	1	86	24	9
1911.....	68	9	99	14	11
Period of fifteen months from 1st January, 1912, to 31st March, 1913.....	104	17	231	147	13
Year ended 31st March—					
1914.....	241	25	577	106	25
1915.....	529	78	467	83	24
1916.....	688	88	346	52	34
1917.....	881	144	147	86	50

Critically considering such a table, however, it has to be allowed from the commencement that any idea formed of the conditions existing in the years for which it is compiled can only be one of extremely general character. Thus it cannot be held for a moment that these data show, in any year, the number of cases actually occurring. A large number of Anthrax cases are never reported even at the present day when the disease has come to be better recognized by stockowners generally. Sometimes such non-reporting would appear to be due to carelessness or neglect; sometimes to a desire to conceal the presence of the disease, but very often it is because the disease has been mistaken for some other conditions. Amongst those for which it has been so mistaken may be mentioned Redwater, Gallamziekte, Blackquarter, Rinderpest, Plant-poisoning, Horse-sickness, Purpura haemorrhagica, and Snake-bite. Bearing in mind, however, that increasing acquaintance with the disease on the part of owners leads to more general reporting, and also that administrative operations involving examination of a larger number of blood-slides from dead animals* have caused more cases to come to light in later years, it cannot be considered that the increase in prevalence in certain of the Provinces will be any more than very roughly in the proportions indicated by the statistics in the table. Considering the statistics by themselves it may therefore be maintained that their value is slight, and that inasmuch as they do not allow us to judge the extent to which the disease has actually been prevalent in any given year, and in how far the increase is real as distinguished from the apparent, they are apt to lead to false deduction. At the same time, however, other collected evidence leaves little room to doubt that the great increase indicated for the later years is a very real one indeed. Such evidence is to be found in the opinion of those controlling the disease. Belief

* As for instance where inoculation against a disease such as East Coast Fever is being carried out in native territory.

in the increase of prevalence is, for instance, to be found stated in all the reports of the Principal Veterinary Officer of the Union subsequent to 1910, not only by the Principal Veterinary Officer himself, but also by the Senior Veterinary Officers of the various Provinces. That this view is the one held by all these veterinary administrators is very clear from statements which may be quoted here in some detail.

Report of Department of Agriculture, 31st May, 1910, to 31st December, 1911.—In the report of the Veterinary Division for 1911 herein contained, the Principal Veterinary Officer remarks:—

“In a former provincial report, I drew attention to the careless manner in which the carcasses of animals dying under circumstances which might reasonably give rise to suspicion of Anthrax, were disposed of, or rather to the manner in which they were not disposed of, particularly in some parts of the Western Transvaal, expressing a view that this continued indifference would probably result in the disease becoming increasingly prevalent.

“Consideration of the latest returns makes it only too evident that this is what is now happening, and not only is it happening in the Transvaal, but in other Provinces as well, and the position in some parts of the Union now appears to point to the necessity for a general inoculation of stock in order to protect them from the ravages of this disease. . . . In the Transvaal during the past year 68 outbreaks have been dealt with by the authorities, in the course of which 98 animals died of the disease. This statement rather appears to indicate that outbreaks of the disease are attended by a trifling mortality, but that is only the case when outbreaks are promptly reported and the carcasses of infected animals are properly disposed of, but when sporadic cases do occur and the carcasses are left lying on the veld, although there may be no immediate mortality these carcasses establish centres of infection which will spring into activity at a later date, and the infection of an area in which an animal originally died will be heightened to such an extent that it will be almost impossible for animals to live in them. Consideration of the outbreak returns at the end of this report will show that nearly one-half of the total number of outbreaks dealt with occurred in the Witwatersrand District, and the exceptionally high contamination of this area is undoubtedly due to the past disinclination of stockowners to report deaths or claim the carcasses of animals found dead within the boundaries of the various municipalities along the Reef, lest they should be called upon to pay sanitary fees for the removal of the carcass. The consequence is that the mine natives frequently act as scavengers and cut up and carry off the meat with a supreme indifference to their own safety and that of the community, for which they sometimes pay dearly. In the Cape Province the position is no better than it is in the Transvaal, 113 outbreaks are recorded, accompanied by a mortality of 409, but the Senior Veterinary Surgeon of the Province gives it as his opinion that this return does not give anything like a correct idea of the number of outbreaks which have actually occurred during the year. In Natal there have been 11 outbreaks and the relatively high mortality which has accompanied them makes it quite clear that these outbreaks were not reported as promptly as they should have been, and that the carcasses of the animals which died in the first instance were not disposed of in a proper manner. In these 11 outbreaks no less than 112 head of stock succumbed to the disease. In the Orange Free State a somewhat similar state of things seems to have existed, because 45 animals are reported to have succumbed in the course of 9 outbreaks, which emphasizes the necessity for greater promptitude in reporting in order that proper care may be taken with regard to the disposal of the bodies of infected animals.”

In his report for the period 1st January, 1912, to 31st March, 1913, the same officer writes:—

“Our records give us good reason for believing that this disease is becoming increasingly prevalent. This must be attributed to the indifference and carelessness displayed by many farmers in dealing with the carcasses of infected animals. . . . This apathy is, perhaps, more marked in those parts of the country in which the disease Gal-lamziekte is reported to be prevalent. In such localities it is customary to attribute all deaths amongst stock to this disease and to dispose of all carcasses with a sublime disregard for the public consequences, and I am satisfied in my own mind that at least some of the farms

which have acquired a bad reputation on account of Gal-lamziekte do not deserve it, and that the mortality occurring thereon is occasionally due to Anthrax. . . . Indirectly we frequently obtain confirmatory evidence in support of this view in the statement so often made by farmers that Anthrax vaccine is a preventive of Gal-lamziekte. . . . In such cases the inference is obvious—the original disease which was arrested by the Anthrax vaccine was, without doubt, not Gal-lamziekte but Anthrax. . . .

“Cape Province: Senior Veterinary Surgeon Dixon comments on the prevalence of Anthrax in Griqualand West, Bechuanaland, and in native areas in the eastern portion of the Provinces. . . .

“Transvaal: In the Transvaal this disease is fairly prevalent in the Witwatersrand and in the Krugersdorp, Potchefstroom, Lichtenburg, and Marico Districts, while sporadic cases have occurred in Waterberg, Bloemhof, Pretoria, and Carolina Districts. . . .

“Transkei: In this portion of the Union 119 outbreaks have been detected, chiefly in the course of smear examinations in connection with the diagnosis of East Coast Fever, and I think it may be taken for granted that although natives know this disease and recognize it they do not report it. . . .

“Orange Free State: Senior Veterinary Surgeon Grist reports that outbreaks of this disease appear to be on the increase. During the year there have been 15 outbreaks, 7 of which occurred in the Kroonstad District.”

Again, in the report for the period 1st April, 1913, to 31st March, 1914, it is stated:—

“There is no doubt whatever that outbreaks of Anthrax are becoming more common every year in all parts of the Union, and in some localities this disease is becoming such a scourge that in time to come, unless general inoculation is carried out regularly, losses from this disease will be very heavy. . . .

“From a consideration of the Cape Anthrax returns I am satisfied that outbreaks of this disease are very rarely reported to the authorities, while the Senior Veterinary Officer expresses the opinion that in native areas probably more animals die from this disease than from any other.

“Cape: In the Cape no fewer than 577 outbreaks of Anthrax have been reported and dealt with, as compared with 197 last year . . . but what excites suspicion that the disease is much more prevalent than is generally believed to be the case, is the circumstance that of this number of outbreaks 436 occurred in the Divisions of East London and Kingwilliamstown, in both of which it is customary to collect blood-smears from all animals dying suddenly and examine them microscopically to make certain that death has not been due to East Coast Fever, and I have no doubt that if a similar line of action were followed in other districts we would find that the position in other districts is in all probability much the same as it is in the two which I have mentioned.

“Transvaal: In this Province the Senior Veterinary Surgeon reports that Anthrax may be regarded as prevalent in Pretoria, the Witwatersrand, Krugersdorp, Potchefstroom, Lichtenburg, and Marico, and that sporadic cases occur in most of the other districts in the Transvaal, but I may say that I am inclined to believe that if we were more fully informed we would probably find that some of the other districts are no better off than those placed in the first category.

“Natal: In Natal 25 outbreaks of Anthrax have been dealt with, but no record has been kept of the number of animals which have died or the number which have been inoculated. Last year the number of outbreaks dealt with was 11.

“Transkei: In native territories 106 outbreaks of Anthrax have come to our notice, in the course of which 251 head have died and 3313 in-contacts have been dealt with. Compared with last year, when there were 119, the number of outbreaks shows a slight falling off, but this is attributed by the Senior Veterinary Surgeon to the smaller number of blood-slides sent in for examination as compared with the number submitted last year, rather than to any slackening off in the prevalence of the disease. In the Engcobo District, in consequence of an outbreak of Anthrax amongst horses in the course of which several died, a large number of natives have had their horses inoculated against this disease.

“Orange Free State: The outbreaks of Anthrax dealt with in the Orange Free State number 25. The most serious of these was one which occurred on the Kroonstad Municipal Lands, where seven head of cattle and eight mules died of the disease before it was checked by inoculation. I do not think this record faithfully reflects the position of the Free State with regard to this disease.”

Finally, quoting the Principal Veterinary Officer's statements in his report covering the year ended 31st March, 1916, we may note the following details:—

"Anthrax: In spite of the amount of attention and comment bestowed upon Gal-lamziekte at farmers' meetings and in the columns of the public press, it is, in my opinion, very doubtful whether the aggregate annual losses from Anthrax is not very much greater than the mortality from Gal-lamziekte, although the former is a preventible disease which can be controlled and stamped out by inoculation.

"In the Cape Province the Senior Veterinary Officer reports that the disease is extending and, as no determined effort is made for its suppression, the losses in time to come will probably be greater than they have been in the past. European stockowners, also natives, are now beginning to use vaccine for the protection of their stock in infected areas, but unfortunately many defer inoculating their animals in such areas until after an outbreak has occurred and their losses are frequently considerable. . . . 346 outbreaks of Anthrax have been reported in the Cape Province during the year, probably only a small proportion of the total number which have actually occurred.

"In the Transvaal, Anthrax is still very prevalent, particularly along the Reef. 688 outbreaks have been reported and dealt with as against 529 last year; 1000 animals have died from the disease; 27,500 head have been inoculated.

"In Natal the number of outbreaks continues to be relatively small—34 in all have been reported; 211 head have died and 5379 in-contacts have been dealt with. The Senior Veterinary Officer stated that stockowners readily resort to preventive inoculation whenever the disease makes its appearance.

"In the Transkei the Senior Veterinary Officer reports that the losses from this disease have not been very heavy—52 outbreaks have occurred, the most serious being one at Ramhlokoana's Location, Matatiele, where 85 deaths occurred. In every outbreak in-contacts were promptly inoculated.

"In the Orange Free State, Anthrax is becoming increasingly prevalent—88 outbreaks have been recorded; over 500 animals have died and 12,763 have been inoculated."

In taking these statements along with the statistics given as sufficing to show the widespread distribution of the disease throughout the country, with a marked increase in prevalence in some parts in late years, some notes in regard to seasonal and local prevalence may next be offered. That in other parts of the world a greater frequency in occurrence of the disease is associated with a certain season of the year, or with certain local conditions depending on the nature of the soil, sub-soil, water-level, and distribution of surface water in the region, is indicated by the writings of most of those who have given attention to the disease. In these writings an "anthrax season" and certain so-called "anthrax regions" are referred to.

Thus Hutyrá and Marek ⁽⁷⁾ state that as soil disease in progressive agricultural countries Anthrax is usually confined to certain localities where the soil is rich in organic material, moist, marshy, or periodically inundated, and there occurs enzootically almost annually among animals at pasture in summer time.

Nocard and Leclainche ⁽⁸⁾, Sobernheim ⁽⁹⁾, and Burow ⁽¹⁰⁾ record similar views, the latter two authors stating that in Germany outbreaks are most frequent in the second and especially the third quarter of the year, the greatest prevalence being noted, according to Sobernheim, from the middle of June to the end of September.

By all of these authors reference is also made to the rôle which the groundwater, through variations in its level, is believed to play. Rapid recession of level, associated with suitable temperature conditions, are regarded as favouring the germination of the spores and multiplication of the bacilli present in the soil. The work of Bollinger, Friedrich, and Pettenkofer is quoted in support of this view, and it is contended that the facts recorded in this connection are capable of explaining why the frequency of the disease is greater in certain seasons, years, and localities than in others.

In America (Delaware), according to Dawson ⁽¹¹⁾, Anthrax occurs under somewhat similar local conditions, the season lasting from March to November, with greatest prevalence of the disease in the summer months.

Grandmougin ⁽¹²⁾ records that in Madagascar one can always recognize Anthrax "seasons," "years," and "regions," and notes that the greatest prevalence is met with, not at the height of the rainy season or in the period immediately subsequent to this, but later, when by progressive drying up of most of the vegetation of the relatively higher portions of the country, the herds are forced to resort for grazing to the lower lying, moist, marshy regions.

In regard to Great Britain, however, McFadyean ⁽¹³⁾—commenting upon a statement of Opperman's to the effect that the occurrence of Anthrax stands in very close relationship to the nature of the soil, especially as regards moisture and temperature—states that no relationship is observable.

Stockman ⁽¹⁴⁾, from a study of the quarterly incidence of the outbreaks of Anthrax in Great Britain over the years 1906 to 1910, comes to the conclusion that a constant and decided drop in the number of outbreaks is to be noted in the third quarter of the year when, it may be assumed, most animals are still running at pasture, while a marked rise is apparent in the fourth quarter, a time when most animals are under conditions of stabling.

In considering the occurrence of Anthrax in South Africa, it is impossible to bring forward any statistical evidence of a special seasonal prevalence on account of defective reporting of outbreaks; nor does the evidence collected warrant any definite assertion in the matter being made concerning the influence of local conditions. At the same time, however, certain general statements appear permissible. Thus as regards season it may be said that in some parts of the country—greater part of the Transvaal and portions of the Cape Province—the disease appears to be more prevalent during the wetter and warmer months of summer and autumn, from about the latter portion of December on to April, than during the other months of the year, while in connection with local favourable conditions the greater prevalence of the disease in the neighbourhood of pans and vleis or marshes, in certain regions, is to be noted.

In support of this certain observations of Hutcheon and Dixon may be quoted:—

Hutcheon ⁽¹⁵⁾ writes as follows:—"As I have already stated with regard to this territory of Griqualand West, these pans or vleis are the principal source of 'Gift-ziekte' or 'Melt-ziekte.' . . . These places are generally safe to graze over during the winter months . . . but great care and watchfulness are required to graze stock there with impunity during the summer months. Especially do such places become dangerous after fine rains have fallen when the grass has sprung up, and the stock are commencing to improve rapidly in condition." Elsewhere ⁽¹⁶⁾ he writes: "Anthrax prevails principally during the summer and autumn months of the year, because a certain temperature is necessary for the growth and multiplication of the organisms upon which it depends. . . . A certain amount of moisture is also necessary for the development of all organic life, and this is well supplied in the meadows, pans, vleis, and marshy places of this Colony." Salt River, Maitland, and the surrounding neighbourhood are specifically referred to, and the abundance of pans and vleis in the districts of Griqualand West, where the disease is prevalent, is also noted. Dixon* at a later period—1915—writing concerning prevalence in the Cape Province states: "Anthrax and Blackquarter are prevalent over a large area of the country; the only portion of the Cape Province where we can declare it is not enzootic is the Karroo. I consider that in this country Anthrax may be termed a seasonal disease, for it appears to be more prevalent under warm, moist conditions, which occur during the summer months. On the other hand, Blackquarter in the Cape Province is more prevalent during the dry seasons. I do not think that soil conditions, unless it be due to moisture, affect the existence of Anthrax to any appreciable extent. The character of the vegetation probably does, seeing that the Karroo vegetation is so different from the grass veld where Anthrax organisms flourish. In Griqualand West the vleis and edges of pans are known to be hot-beds; this I think is due to these places being moist and therefore most favourable to the growth and multiplication of Anthrax bacilli. In this Province Anthrax is always prevalent in areas occupied by natives.

* Private communication.

I have no doubt this is chiefly owing to the improper disposal of affected carcasses, and when the warm moist conditions appear, the disease becomes a regular scourge."

That, however, there are other local conditions influencing prevalence which have to be taken into account is indicated by certain observations.

Thus, Viljoen,* writing of Vryburg District, states that

"Anthrax was very prevalent in this district in 1916, much more so than in 1917. In 1916 the grass was comparatively very short and the possibility exists that by close grazing on short veld the animals were more likely to pick up Anthrax spores from the soil. In the latter half of 1916 and during 1917, heavy rains fell throughout the district, the result being that the veld was much higher and more abundant than it has been for some years. It is possible, therefore, that the fewer cases of Anthrax in 1917 is to be attributed, to some extent, to the fact that there was no need of close grazing by the animals."

It may be noted here that an explanation similar to this has also been offered by Stordy⁽¹⁷⁾ to account for a sudden increase of the disease in certain districts of British East Africa during 1914-1915. It has also been suggested that in certain parts of South Africa Anthrax may be transmitted through the medium of certain flies. Hutcheon's remarks in regard to the part played by "horse-flies" (*Hippoboscidae*) in spreading the disease in Griqualand West have already been noted in discussing the general prevalence of the disease, and I am informed by Mr. Montgomery, who investigated an extensive outbreak occurring amongst equines in the Boshof district this year, that the belief that the disease was largely spread in this way was one expressed by many owners. In this outbreak, which occurred during a relatively dry period of the year, the mortality occurred among cattle and sheep in only a very few instances, even although in certain cases these animals were grazing on the same farms with equines which became affected. Heavy mortality occurred principally amongst horses, but also amongst mules and donkeys, and this mortality continued on many farms where vaccination was performed. Whether the suggestion that Anthrax may be naturally transmitted in South Africa through the agency of hippoboscid flies can be supported by direct experimental evidence remains to be seen, no experiments on the point having as yet been carried out. Recent observations⁽¹⁸⁾, ⁽¹⁹⁾, ⁽²⁰⁾ in other countries indicate that flies can play a rôle as vectors of the disease, and the point has been raised here in order to suggest that if this is the case the prevalence of the disease in some parts of the country will have to be correlated with climatic and other conditions favouring the insects specially concerned. In summing up, it may be again emphasized that the evidence which we so far possess in regard to both seasonal or local prevalence is of very incomplete and fragmentary character; and that there exist many lacunae in our knowledge which it is hoped further experimental work and more extended observation will ultimately fill in.

3. ANIMALS AFFECTED.

In general, the disease is most common amongst cattle, but in some areas, notably in Griqualand West, British Bechuanaland, and this year in the Boshof district, it has been known to produce a large

* Private communication.

mortality almost exclusively amongst horses, mules, and donkeys. In equines that form of the disease associated with swellings on different parts of the body has been described as occurring very frequently, Hutcheon⁽¹⁵⁾,⁽¹⁶⁾ stating it to be the form most frequently seen in the horse in Cape Colony. According to his description, these swellings are met with most frequently on the lower parts of the chest, abdomen, sheath, and around the anus: "*Some of the swellings are small and almost unnoticeable, while in other cases they cover the whole of the under-surface of the chest and abdomen. In some cases the head is enormously enlarged, the swellings are somewhat hard to the touch, and when opened emit a clear, amber-coloured fluid. The submaxillary and large lymphatic glands are generally slightly swollen. The pulse is quiet and weak, but the heart's beat is struggling, and the temperature high, from 105° to 107° F. This form of the disease has been at times mistaken in equines for purpura-haemorrhagica. It is also seen at times in cattle, and is the form referred to by many farmers as 'Gift-ziekte.'*" Sheep, goats, and pigs are also affected, but to what extent, even relatively, it is difficult to estimate, as it is only when any of these animals die in large numbers that outbreaks are reported. At times it has been observed that when animals of different species have been grazing together over the same veld only the animals of one species have been affected. With cattle and sheep grazing on the same veld the disease has, for instance, appeared only amongst the sheep, and with horses and cattle grazing under the same conditions the equines have been almost exclusively affected. Very young animals may be affected, and cases have been reported where thriving lambs a few weeks old have died during an outbreak in numbers almost equal to those occurring in the adult sheep; and also where some young sucking foals have died shortly after the deaths of their dams.

It may also be here remarked that two cases have been reported where dipping would appear to have had some effect in causing the disease, already present on the farms concerned, to manifest itself to such a degree shortly after the operation as to make one suspect that a "latent-infection" had been developed in evident form, possibly by lowering the resistance of the animals. In one of these cases, reported by Andrews,* the animals affected were cattle, while in the other the outbreak occurred among sheep after the first dipping for scab; in both cases the disease showed itself in unusual degree within about twenty-four to forty-eight hours after the dipping. What would appear to be recovery from infection has also been recorded, animals which during an outbreak showed an unusually high temperature—106° to 107° F.—with other symptoms of bodily disturbance, later returning to normal condition. Anthrax is also met with in the dog, in the ostrich, and in man. In view of the very high immunity possessed by most birds its occurrence in the ostrich under natural conditions is a matter of unusual interest; it therefore seems justifiable to deal with it in some detail.

Anthrax in the Ostrich.—Henning⁽²¹⁾ appears to be the first to suspect the occurrence of Anthrax in the ostrich, the idea occurring to him during an investigation into a serious mortality amongst the birds running on a farm in the Caledon District of Cape Colony in 1894. On this farm about fifty full-grown ostriches, constituting

* Private communication.

nearly seventy-five per cent. of the troop, had died within a few weeks. All of these birds had been in very good condition, and had only shown unusual symptoms a couple of hours before death, the symptoms noted in each case being those of great dullness and prostration. No bird that had been noticed to be ill had recovered.

Post-mortem examinations were made in eight cases, and Henning gives a general description of the findings, which allows of recognition of similarity with the lesions later found in other birds which undoubtedly died from the disease. He specially refers to the fact that neither round-worms nor tape-worms nor any other parasites were met with in these birds, and that all which died had been in very good condition, the subcutaneous and sub-epicardial fat being very well developed. In the blood examined immediately after death, there were apparent "a great many microbes similar to the *Bacillus anthracis*, but larger than these usually appear." One sheep and two fowls were inoculated with spleen from one of the dead birds, and of these inoculated animals the sheep died after twenty-four hours. Unfortunately, Henning was unable to complete his investigations, but as a result of what he had observed he formed the opinion that this outbreak was most probably one of Anthrax. He further adds that he was able to convince himself, by examination of a cow that died, that the farm was one on which Anthrax was present; also, that some one who had made some post-mortem examinations before his arrival "caught a very dangerous blood-poisoning which made her unfit for any work for months." In 1905 Robinson⁽²²⁾ met with the disease on a farm in the Mossel Bay district of Cape Colony. Horses, cattle, and ostriches were affected, and in his report on this outbreak he mentions that he was then making experiments with the surviving birds to determine whether the Pasteurian vaccination would be as effective in protecting them as it was in the case of other farm animals.

Robertson⁽²³⁾ in 1908 also reported a case of Anthrax in the ostrich. This case occurred in a full-grown hen, one of seven birds then kept at the Grahamstown Laboratory. In 1911 a somewhat serious outbreak of the disease occurred amongst the ostriches in the Oudtshoorn District of the Cape Province. Blood-smears from one of these birds were submitted to the Laboratory at Grahamstown by Mr. Elley, M.R.C.V.S., who suspected the disease, and the diagnosis was confirmed by Walker⁽²⁴⁾. At this time Elley showed that by inoculating with the blood of one of the dead ostriches, Anthrax could be produced in the ostrich chick, boer-goat, and Africander sheep; while Walker proved that by taking a strain of Anthrax derived originally from a blood-smear taken from a dead bovine, and passing this through the guinea-pig and sheep it was possible, with the blood of this last animal, to set up the disease in the adult ostrich. Carougeau⁽²⁶⁾ also records certain cases of Anthrax met with in ostriches in 1910 in Tulear, Madagascar, by Messrs. Schiler and Beny, and mentions that the blood and peritoneal serous exudate of one of these birds, inoculated into rabbits, caused death from Anthrax. As Theiler notes, the Anthrax of the ostrich and of the mammal would, therefore, appear to be identical. The symptoms and post-mortem appearances of Anthrax in the ostrich, as noted by those who have come in contact with the disease in birds under natural conditions, have been collected by Theiler⁽²⁴⁾ in a bulletin "Anthrax in the Ostrich," and may here be repeated for the benefit of those not already acquainted with that publication.

"Peracute form.—This is the form most forcibly brought to notice, because it invariably ends in death. In natural wild cases the birds are generally found dead in the morning, or in a moribund condition, comatose, paralysed, and evidently insensible. Death from Anthrax may occur very rapidly. The birds may have been seen feeding and in every respect looking the picture of health, and a few minutes afterwards are found dead, or they may have been seen to be taken convulsively with staggers, lying down on the side, sparring convulsively with the legs, the neck spasmodically twisting and untwisting. Mr. Robertson who was able to watch one of these birds, states that the feathers became erect, the whole body was shaken with tremors, and the bird died with an outstretched neck. Farmers finding the birds dead either attribute the mortality, in the case of chicks to poison, or in adult birds to violence. Mr. Robertson informs me of an instance where 27 two-month-old chicks were shut up at night and found dead the next morning, having contracted Anthrax probably by picking up the infection from the blood dripping off the skin of a sheep which had been hung up.

"Anthrax fever.—Attention has been drawn to this by a farmer who, some years ago, had a severe outbreak of Anthrax among his birds, the diagnosis of which had been confirmed by microscopical examination. In addition to the apoplectic deaths which occurred first, quite a number of cases were noticed where the birds showed symptoms of a general illness, not feeding for one or more days, resulting in death or more generally in recovery. So far we have had no microscopic examination of the blood of such birds, and we do not know whether in Anthrax fever the bacteria are present or absent from the circulation. It is possible that in Anthrax fever high temperatures will be registered, but so far experience has shown that in the case of inoculated Anthrax of the apoplectic type the temperature taken before death was not so high as one would expect. The normal temperature of two seven-month-old healthy chicks taken in the rectum during a period of thirteen weeks, varied from 102° F. in the morning to 103° F. in the evening.

"Post-mortem lesions.—These refer to apoplectic cases. The animal is frequently in the best of condition. Rigor is usually absent. The blood which escapes from the cutaneous and subcutaneous veins whilst skinning is performed is usually dark in colour and coagulates very badly; the veins themselves are filled and distended. In the pleuro-abdominal cavity a smaller or greater amount of liquid can be found, which is occasionally reddish discoloured. Henning found this quantity to be considerable, amounting from one to five bottles. On the pleura of the ribs, on the pericardium, the peritoneum, and the mesentery quite a number of blood-spots varying in size from very small (petechiae) to that of a sixpence (ecchymoses) or diffuse patches (haemorrhagic infiltrations) can be seen, larger patches at the same time showing gelatinous infiltration (suffusion and sugillation). The liquid in the heart-bag (pericardium) may also show a considerable increase. The lining of the outside of the heart (epicardium) as well as that of the cavities (endocardium) usually show similar blood-spots to those mentioned, or extensive haemorrhagic infiltrations; these latter were found by myself in a bird thirty-five years of age at the base of the main artery (aorta), extending in course for a distance of one or two inches. The heart muscle itself may show haemorrhagic infiltrations. The blood, generally speaking, is deep black, tarry, and either partially or not at all coagulated. There are, however, cases on record where coagulation has taken place. The lungs as a rule show nothing unusual, at least nothing typical; they may be engorged with blood (hyperaemic) and on section froth may escape from the cut surface. Froth may also be present in the windpipe or trachea (oedema). The stomachs (proventriculus and ventriculus) are usually found in normal condition. Most frequently both contain fresh food, and in the former is the usual thick deposit of mucus in which smaller or greater numbers of wire-worms are found embedded. The entrance to the small intestines (pylorus) may be swollen and reddened. The small intestines (duodenum, jejunum, and ileum) are as a rule in a state of acute hyperaemia, viz., a more or less intense infiltration with blood is noticeable throughout the mucosa, which shows a swelling and uniform reddish discoloration; an increased amount of mucus may also be found. In other instances the hyperaemia is not uniform but irregularly distributed in smaller and larger areas, in patches and streaks throughout the length of the intestines. This state of affairs can sometimes be noticed when inspecting the external side (serosa) of these parts. The acute inflammation may be found to be still more strongly pronounced in the colon, the mucous membrane being even thicker, the surface corroded, and the submucosa gelatinous, or the whole length is studded with haemorrhages or softened or contains necrotic patches.

The contents of the colon may be mixed with blood; they are either in the form of dry pebbles or semi-fluid. The liver appears in a state of acute congestion, it is enlarged, bluish in colour when examined from outside, and dark when cut into, blood freely escaping. The spleen may in some instances be found to be of normal size and condition. Usually, however, it is more or less enlarged and of a dark colour; when cut into the capsule distends at once, the pulp protruding over the surface. The kidneys may be normal or in a state of congestion, appearing darker and more friable. The blood taken from the dead body shows the characteristic *bacillus anthracis*."

Anthrax in Man.—In the human subject many cases of anthrax have been known to occur. The majority of these have been met with in natives who contract the disease in skinning or cutting up the carcasses of dead animals, or after eating their flesh. But many cases have also been noted in white persons who have perhaps carelessly handled anthrax carcasses in opening them to attempt to ascertain the cause of death, or who may have been engaged in handling hides derived from infected animals.

Through the kindness of Dr. Arnold, Medical Officer of Health for the Union, the statistics available as to the number of cases of Anthrax occurring in human beings have been furnished for this article, and are given in the table below:—

Cases of Anthrax in Human Beings notified in Years 1913 to 1916.

	1913.	1914.	1915.	1916.
<i>Cape Province—</i>				
White persons.....	2	5	12	10
Coloured persons.....	4	4	17	10
<i>Natal Province—</i>				
White persons.....	—	—	2	2
Coloured persons.....	2	—	34	6
<i>Transvaal Province—</i>				
White persons.....	3	14	22	13
Coloured persons.....	8	10	19	18
TOTAL—				
White persons.....	5	19	36	25
Coloured persons.....	14	14	70	34

Return of Deaths among Europeans resulting from Anthrax, Years 1912 to 1916.

	1912.	1913.	1914.	1915.	1916.
Cape Province.....	—	—	1	—	—
Natal.....	—	—	—	1	—
Transvaal.....	—	1	—	—	2
Orange Free State.....	—	—	1	—	—
	—	1	2	1	2

In explanation of these statistics, however, the Acting Under-Secretary for the Interior states:—"As regards deaths amongst coloured persons amongst whom such deaths of course mostly occur, the Department is not in a position to give any reliable information. In the Provinces of Natal and the Orange Free State the causes of death amongst coloured persons (natives) are not registered; in the Transvaal only deaths in certain urban areas are registered, whereas such deaths mostly occur in rural areas; in the Cape Province the registration of deaths amongst natives is extremely unreliable, in certain parts only about 6 per cent. of such deaths being registered. The disease is not 'notifiable' in the Orange Free State Province."

In view of these conditions it is, therefore, most probable that the number of cases occurring in the human subject is very much greater than that indicated in the above tables. The cutaneous form of the disease (malignant pustule or carbuncle) appears to be the one more commonly seen in man, but cases of the internal or primarily intestinal form have also been met with. That these latter are not more common is probably due to the fact that the spore form of the organism is infrequent in the flesh and other parts of the infected carcasses concerned, and that the bacillary form present is destroyed either in the process of cooking or by the digestive juices. Many older settlers, however, explain the relatively low incidence of death amongst natives on the hypothesis of the protective action of certain medicinal herbs which find their place in the native pharmacopoeia.

The Principal Veterinary Officer (Union) in his report for the year 1912-13, remarks: ". . . . I think it may be taken for granted that although natives know this disease, and recognize it, they do not as a rule report it, and carcasses of animals dying from Anthrax are frequently cooked and eaten, generally with the addition of certain herbs which are supposed to protect the consumer against the possible consequences of his meal. Occasionally those who cut up the carcasses, or who take part in the subsequent feast, succumb to the disease, but these accidents occur less frequently than might be expected." That these herbs possess any value in the respect claimed is not a belief to be accepted without very convincing tests, and, so far as one is aware, none have ever been carried out.*

4. SPREAD OF INFECTION.

In the spread of the disease under South African conditions the part played by human agency assumes very great importance through the existence of the native population. The majority of natives

*Smith in his "Contribution to South African Materia Medica, Lovedale, 1895," mentions some of the herbs used by natives in the treatment of "Anthrax" or "Milt-ziek" poisoning, and gives the following list: *Blepharis capensis* and *Crobbra cirsoides*—Kaffir, *ubu-Ilungu besigawu*. *Cluytia hirsuta*—Kaffir, *ubu-Ilungu bedila*. *Matricaria nigellaeifolia*, D.C.—Kaffir, *um-Hlanyane womlambo* (river-wormwood). *Xanthoxylon capense* (Harv.)—Knobwood, Wild cardamom—Dutch, *Paarde ptaam*—Kaffir, *um-Nungumabele*. *Teucrium africanum*—Dutch, *Padde klauw*—Kaffir, *an ulu-Ilungu benyushu*.

These plants he describes as used in decoctions in different ways, some, e.g. *Cluytia*, being an "antidote for Anthrax," and also a disinfectant for "milt-siek meat," while others are "antidotes" alone, e.g. *Matricaria nigellaeifoliae*, or "disinfectants" only, e.g. *Xanthoxylon capense*. Blain is also quoted in an abstract appearing in the *Cape of Good Hope Agricultural Journal*, 1897, as having used certain preparations in the treatment of one case of malignant pustule. Here, however, the author could only assert that recovery followed the use of the plant extract, and does not essay any explanation of its action.

consider that whatever a bovine or sheep may have died from the flesh serves a better purpose in being consumed by them than in being buried or burned. It therefore frequently happens that the carcass of an animal dead from Anthrax is skinned by them and eaten; and if the outbreak should affect a large herd of cattle so that a number die in a locality where a number of natives are gathered together, as in reserves or locations, many carcasses may meet this fate before official action comes into force.

The difficulties of enforcing stock disease regulations under such conditions, with a relatively limited staff of officials to administer and police large areas of country will be apparent, and hence it may be that the practices in question may continue in spite of repeated intervention, and ultimately only with greatest vigilance be prevented. Also, the matter as a rule does not end with the consumption of the meat in the immediate neighbourhood of the carcasses. Frequently the infection has the opportunity of becoming spread for a considerable distance from the site where these carcasses lie, through the carrying away of portions of the meat to huts or kraals in the vicinity. This meat may be cooked and eaten in a more or less fresh state, or may be dried as biltong and then used for consumption at a later date. This description of what may occur under rural conditions may also apply to urban areas. The Principal Veterinary Officer (Union) refers in this direction in his annual report for the year 1912-13 so much to the point that his remarks may be quoted verbatim:—

He writes:—

“In the Transvaal this disease (Anthrax) is fairly prevalent in the Witwatersrand and in the Krugersdorp, Potchefstroom, Lichtenburg, and Marico Districts, while sporadic cases have occurred in Waterberg, Bloemhof, Pretoria, and Carolina Districts. In all cases that come under the notice of the Department, disinfection is insisted upon and preventive inoculation is now being carried out on a large scale. The area in which greatest trouble has been experienced in dealing with Anthrax has been the Witwatersrand, and this has been due to the somewhat peculiar local conditions.

“In that part of the Transvaal, which consists largely of small townships, large numbers of milch cows are kept by private owners and others which are permitted to graze over the unfenced veld during the day and are brought up in the evening for milking, and when one of these animals happens to die suddenly, whether from Anthrax or any other cause, the owner not infrequently abstains from reporting his loss to the authorities lest he be called upon to pay the sanitary fees charged for the removal of the carcass. As a consequence such carcasses are removed piecemeal by natives from neighbouring mine compounds, and when one of these animals happens to have died of Anthrax and the blood and viscera are scattered broadcast over the veld, the inevitable result is a succession of cases of Anthrax amongst the cattle grazing in the locality and occasional deaths amongst natives from malignant pustule, and the ‘old subscriber’ picks up his pen and writes to his newspaper to inquire what the Government Veterinary Officer has been doing and why he has not taken steps to prevent such mortality, when the harm has really been done before the Department has been advised or has had any opportunity of dealing with the outbreak.

“On account of the slackness on the part of owners in reporting the loss of their animals, special warnings have been published in the local Press and in the *Agricultural Journal*, directing the attention of all concerned to the dangers resulting from their want of care, and special regulations under the Stock Diseases Act, requiring owners to report all deaths occurring amongst cattle in the Witwatersrand to the authorities, in order that timely information may reach the Department, but even when this is done unless the carcass is looked after sharply the natives frequently carry it away; and I remember very well in one particular instance when the local veterinary officer went to the spot where an animal had died, in order to ascertain what it had died of, all that was left of the carcass was a portion of the hide, and from that remnant the officer obtained a smear by which he ascertained that Anthrax had been the cause of death.”

Even when the carcass is not opened up for the purpose of eating the flesh, it is often skinned for the sake of the hide. On other occasions it is opened in order that the owner may make a post-mortem examination, and it is stated, with regrettable frequency, by owners, either verbally or in correspondence, that they were convinced that certain cases of death were due to Anthrax on the evidence of the appearance of the spleen. From the carcass infection may also possibly be spread through the agency of dogs, pigs, and also, perhaps, wild carnivores and carrion feeding birds (jackals and vultures). With respect to the two latter classes of animals, definite statement has to be specially qualified until further observations on the point are available, although meanwhile it appears justifiable to consider them with the greatest suspicion as agents assisting in dissemination of infection. The experiments of Morris (*l.c.* 20) and others go far to support suspicion at least under conditions where the organism in the infected material is present in the spore stage. Cattle may also feed on portions of the carcass, while sheep and goats may devour the contents of the stomachs. Hutcheon* records deaths from Anthrax, especially in sheep, after feeding on such material, stating it as his belief that the disease had been so acquired. Then, again, the infection may be spread through the bones of the dead animal, if these are ground up as bone-meal, and, as sometimes occurs, this material, without having undergone sterilization, is used either as a constituent of the "lick" so commonly used for different kinds of stock, or else as a "soil-fertilizer." In one instance bone-meal infected with Anthrax was detected in the laboratory by means of inoculating mice.

The part played by the infected skin or hide is worthy of special attention. The skin may be removed as a preliminary to the dissection of the carcass if this is to be eaten; or, if the owner believes that trouble with the administrative authorities may be avoided by burying the carcass, he may still try to evade total burial by first removing the skin. The skinning by natives of a carcass exhumed after burial has even been reported. After flaying, the skin may perhaps be kept on some part of the owner's premises—possibly spread out on the ground in the vicinity of the homestead to dry, or hung up in some shed or building or on the wall of a kraal, i.e. in places where living animals of one species or other may be kept. It may even be placed in some collection of water, such as a pan or dam from which healthy animals may later have occasion to drink, or it may be concealed by a native owner in some bush, kept until the death from Anthrax has been forgotten, and then traded as the skin of an animal recently killed to provide food for some festive occasion. Parenthetically it may be remarked that the killing of cattle for food by the natives is unusual save when necessitated by some special celebration, and that

* Report of the Colonial Veterinary Surgeon, Cape of Good Hope, for the year 1884, Capetown, 1885.

In the same report another reference to the carnivorous propensities of sheep and goats is given by Hutcheon in the description of a visit to a farm in Griqualand West. On this farm 200 cattle had died from Lamziekte and Stijfziekte, and Hutcheon writes: "He took me to the place where the principal portions of these animals' carcasses had lain close to the kraals where his sheep and goats were kept, and I could discern only the marks where these carcasses had been. Every portion of the carcasses had been devoured by the sheep and goats and remaining cattle."

the bovine skins which they have to dispose of are more usually those of animals which have died from accident or disease than those of clean and healthy animals slaughtered specially for consumption. In dealing with the skin, the immediate local danger, both to stock and to human beings, is obvious enough, but with the trading and subsequent transport through the country comes the possibility of conveyance of infection to very remote parts, and the chance of fairly widespread dissemination of infective material en route. Collected on the wagon of the travelling trader—who usually moves from farm to farm or from location to location until he has a consignment large enough to forward to his business centre—such skins, lightly salted as they often are, may be set out with others on the veld to dry, and so infect the ground where they are laid. The drying process naturally takes place at some convenient halting stage, a recognized “outspan” in the neighbourhood of a “store,” and it frequently happens that other animals come, in the absence of the trader-owner, to chew these skins or lick the salt from them, and so contract the disease. The suggestion that certain unscrupulous individuals trading in this way are not altogether averse to allowing animals to acquire infection in the hope that their skins will later be offered for sale, has even been made, and although it is not one acceptable to the normal moral sense, it is a contingency which the administrative official has to bear in mind. In regard to the danger of the hair or wool derived from such skins to those dealing with such products at their destination, the Principal Veterinary Officer of the Union, in his report for the period May, 1910, to December, 1911, writes as follows:—

“Not only do those who handle the carcasses of animals dying from the disease suffer, but those who handle South African produce in places beyond the sea are endangered by the want of care on the part of those who recklessly forward contaminated material to overseas markets. Only recently reports have reached the Department from the Bradford Anthrax Investigation Bureau of the discovery of Anthrax spores in bales of blood-stained South African mohair and wool, and if greater care is not exercised in time to come the possibility of South African mohair being infected with Anthrax is bound to prejudice buyers and lower the market price to a very marked degree.”

Finally, the reims made from infected skins may be mentioned as means through which the disease may be conveyed; and one case may be recorded in which washings from reims submitted for examination were proved to contain the Anthrax spores. The part that the sick animal may play in disseminating infection through the medium of its infected discharges must be remembered. In addition, however, infection may be spread from it by such practices as the opening up (in attempted treatment) of Anthrax swellings appearing on the body. As to the probable fate of the spores, which under suitable developmental conditions will be formed in the soil wherever infective material has been deposited, it may be that these remain *in situ* to be floated up later from the superficial soil layers by light rains—as Burow suggests—and then be ingested; or it may be that they are taken up in drier seasons by animals grazing more closely than usual and so ingesting some of the infected soil with the herbage. It may also happen that spores are washed by heavy or torrential rains to more distant places and then find their way into pans, dams, vleis, or at times into rivers and later into irrigation streams led from these. Again, when in soil which has become very dry and pulverant either on the veld or in dried-out pans or dams, the dust storms so prevalent

at certain times may possibly be a means of transporting spores for a considerable distance.

In regard to water transport, the remarks of Theiler ⁽²¹⁾ concerning his investigations into the outbreak of Anthrax amongst ostriches in the Oudtshoorn District in 1911 may be quoted:—

“For many ages Anthrax has been considered to be a disease of the soil, and its spread along river beds has been connected with floods and inundations. The development of the ostrich industry in Oudtshoorn and other places was principally due to improved methods of irrigation, and it is possible that the introduction of irrigation is in some instances responsible for the advent of Anthrax and in other cases of its spread. It has been brought to my notice that in a valley where a number of ostrich farms are adjoining each other and which of necessity are all irrigated by the same river, the disease, which was noticed first at the top farm, was also noted on the farms below. It must not be forgotten that Anthrax can also be spread by foodstuffs off an Anthrax farm, which might account for its mysterious appearance on certain places.”

In considering more particularly the infection of dams, pans, and vleis it may be recalled that, under the heading of “Prevalence,” such places have been characterized as “hotbeds” of the disease. The manner in which spores may be carried into pans or dams has just been referred to, but in addition to this transport of infection it must be remembered that pans and dams, as watering places for stock, may be directly infected by animals coming to them to drink and dying in them or in the mud around their edges; while in vleis infection may be maintained through the deaths on them of animals tempted by the vegetation they offer.

It may be emphasized that belief in infection of such places does not rest only on speculative grounds, but is supported by experimental evidence. In the experiments of Robertson and Andrews,* of this Division, samples of suspected water or mud were heated to about 70° C. for a time sufficient to kill the bacillary forms of organisms, and then examined for the presence of the Anthrax bacillus. Robertson was successful in tracing its presence in a sample of water from “a very shallow dam with an extensive catchment area,” and also in three out of four samples of mud taken from “a small dried up vlei which was supplied by a sluic from a camp where many cases of Anthrax had occurred.” With five samples of water from other places results were negative. Andrews was also able to isolate the organism in the water of a dam on a farm where Anthrax was present. That the infection of these places must have been a very heavy one has to be admitted, since the quantity of material used for actual examination was extremely small; but it is interesting to note that the presence of the organism has actually been demonstrated in dam water.

Before concluding this section two other points may be briefly dealt with—the action which certain foods may have in favouring infection and the possibility of insect transmission. As to the first, attention may be called to an observation made by Spreull,† who, investigating an outbreak of Anthrax in the Bedford District of Cape Colony in 1904, formed the opinion that natural inoculation occurred in a large number of cases through the irritated and abraded mucous membranes of the mouth, produced by the prickly-pear on which the animals were fed. It is stated it was only on those farms in the area

* Private communications.

† Report of the Colonial Veterinary Surgeon, Cape of Good Hope, December, 1904.

which were overrun with prickly-pear that the disease spread. In regard to the second point, the reader may be referred back to Section 2, where this question was specifically discussed, and we may merely repeat here that since Schuberg and Böing have shown that under experimental conditions the transmission of Anthrax from dead or sick animals to healthy ones by *Stomoxys calcitrans* is possible, and since Mitzmain has proved that infection from the sick animal may be conveyed by *Stomoxys calcitrans* and *Tabanus striatus*, the belief that in South Africa hippoboscids, and possibly other blood-seeking diptera, may act as vectors of the disease will probably be substantiated by future investigation.

5. DIAGNOSIS.

In the ordinary routine laboratory diagnosis of smears submitted from the field, staining by the Giemsa method is practised; McFadyean's methylene-blue reaction is also made use of at times. In the examination of materials such as blood in quantity, bones, bone-meal, water, reims, etc., the usual cultural and small animal inoculation methods of diagnosis are resorted to.

The Ascoli thermo-precipitin reaction has so far not come into use, owing to present pressure of routine work, but this method will probably find application in the future.

6. CONTROL OF THE DISEASE.

(a) *Regulations dealing with Control and Suppression.*—Prior to 1910 Anthrax was dealt with under the various Colonial Stock Diseases Regulations then in force in the various Colonies, now Provinces, within the Union. In general, the methods then used in coping with outbreaks were those of short quarantine and movement on to a clean area of the animals amongst which the disease had appeared. When the outbreak occurred in a herd or a flock on a farm immediate movement on to a clean portion of the same farm was allowed, and if the animals moved then remained free from the disease the quarantine was removed after about a week. Occurring amongst transport or other animals moving on the road the case was dealt with, allowing the animals to move a short distance from the place where the disease first appeared and keeping them here for three or four days. If no further cases then occurred the animals were allowed to proceed on their journey.

In the Cape Colony Pasteurian vaccination was also used to some extent, and for some localities was strongly recommended although not insisted upon. In the Transvaal and Orange Free State its use in earlier years was not recommended owing to the then apparently limited incidence of the disease. The Transvaal Stock Disease Regulations, drawn up under Ordinance No. 17 of 1902, provided for the disposal of the carcasses of animals dying of Anthrax, and directed that these should be burned or buried intact.

In regard to the other Colonies, however, Theiler and Gray, writing in 1906, state:—

“None of the Colonial Stock Regulations, save those of the Transvaal, indicate what measures shall be taken for dealing with the carcasses of animals dying of Anthrax, but the matter is left to the discretion of the authorities in whose hands the administration of the general stock regulations is placed, which

is somewhat unfortunate, as there is invariably a considerable diversity of opinion amongst laymen as to what constitute the most efficient and satisfactory steps to take for disposing of Anthrax carcasses, and the disinfection of the place where the body has lain.

"The Transvaal regulations direct that the carcasses of animals dying of Anthrax shall be burned or buried intact. . . . In addition to directing the burning or burial of the carcass, the Transvaal law also prohibits the bleeding of any animal supposed to be suffering from Anthrax by any one save a veterinary surgeon, and provides that the excreta, discharges of infected animals, and litter contaminated by these shall be burned or buried, and for the disinfection of the place where the animals have lain."

(b) *Preventive Vaccination.*—The existing regulations dealing with the disease are those made under Act No. 14 of 1911, "To consolidate and amend the Laws in force in the Union for the Prevention of Disease amongst Stock." In these regulations (which are furnished in full as an appendix) provisions are made that the owner shall cause the carcass of any animal that has died, or is suspected of having died, of Anthrax, and also any infective excreta, litter, or discharges to be destroyed by burning or burial; and in addition it is laid down that it shall be the duty of the owner or person in charge of any animal which has been in contact with an animal affected with Anthrax to cause or permit such animal to be inoculated at the discretion of the Principal Veterinary Officer. Owing to the difficulty, however, of compelling owners to comply with the regulations providing for the destruction of infected carcasses and infective materials, it is upon preventive inoculation that main reliance has come to be placed in dealing with all serious outbreaks. With the increasing prevalence and spread of the disease the practice of preventive vaccination has therefore been very greatly extended, and up to December, 1915, the vaccine exclusively used was that obtained from the Pasteur Vaccine Laboratory, Paris. The vaccines so obtained were the usual two types supplied by that laboratory. One of these, which we shall refer to as "double-vaccine," consists of two cultures corresponding in degree of attenuation with the Pasteurian first and second vaccines, and is injected with a 12 to 18-days' interval between the weaker first and the stronger second. The other, which we shall refer to as "single-vaccine," is one whose virulence lies between that of the usual first and second Pasteurian vaccine, and in its use only one inoculation is necessitated.

Towards the end of 1915, however, the conditions resulting from the war rendered it impossible to depend upon regular supplies from Paris, although the demand for vaccine was increasing in an unprecedented fashion. The rate of increase of demand may be illustrated by the following table which shows the total quantities of Anthrax vaccine issued yearly by this laboratory from March, 1911, to March, 1918:—

<i>Year.</i>	<i>No. of doses issued.</i>
1911—1912	30,100
1912—1913	65,200
1913—1914	99,325
1914—1915	190,605
1915—1916	399,680
1916—1917	696,850
1917—1918	509,125

On account, therefore, of the rapidly rising demand—and also because it was thought futile to meet the position by keeping a reserve

supply of material on hand in face of the advice that the material should be used in as fresh a state as possible—the expedient of preparing and issuing vaccines made from the imported attenuated strains was adopted in December, 1915. Since then local preparation has been maintained, the writer taking over the preparation of this material from Veterinary Research Officer Andrews in April, 1916. In July, 1916, the issue of the imported vaccine was almost completely discontinued, and it may be noted that the amount of locally prepared material issued between then and the present time (July, 1918) amounts to nearly half a million doses of the double vaccine, and well over half a million doses of single vaccine. In the ensuing text distinction will be made between material so prepared and the imported vaccine, by referring to the former as “Locally prepared” (L), and the latter as “Imported” (I). During the period April, 1916, to January, 1917, owing to difficulties encountered in obtaining supplies of suitable bottles and corks and also because experience up to that time did not markedly contra-indicate its general use, the only vaccine prepared and issued locally was the single. Towards the end of 1916, however, certain post-vaccinal accidents, to be mentioned later, were reported to follow the injection of this material into horses and cattle, and the issue of the double vaccine for general use was therefore reinstituted in 1917. From that time the single vaccine has been issued for the inoculation of cattle only, and then only when specifically demanded. In the preparation of the vaccine locally, batches of about twenty to thirty thousand or more doses are made at a time. The seed cultures consist of 36-48 hour agar-slant cultures of the attenuated organism, and from these the growth is washed off by means of sterile broth introduced by a pipette. The emulsion thus obtained is then inoculated into a slightly alkaline peptone salt bouillon prepared from beef in the ordinary way, but diluted four times with distilled water. The flasks containing the medium are half filled and plugged with wool through which two fairly narrow glass tubes pass. One of these is relatively short, projecting into the flask only a short distance below the plug, and serves for pipette inoculation of the flask with the emulsion obtained from the seed cultures. The other longer tube reaches to the bottom of the flask, and is bent outside at an angle of about forty degrees. Its outer end, more or less at the level of the mouth of the flask, is fitted with a rubber tube joined to a glass end-piece, which is drawn out and sealed. In the process of bottling, this end-piece is passed through an inverted glass funnel, the end broken off under the usual conditions of sterility, and the liquid then removed by siphoning.

After inoculation the flasks are incubated at 37° C. for a minimum period of forty-eight hours. The vaccine from one of the flasks is then bottled and cultures taken for the small animals virulence tests. These cultures are grown in broth similar to that contained in the flasks and injected subcutaneously when about forty to forty-eight hours old into white mice, guinea-pigs, and rabbits. If this test proves the vaccine to possess a suitable degree of virulence, the remainder of the flasks are then bottled. The bottles of vaccine are then placed in the incubator at 37° C., in order to test the bacterial purity of their contents, and then transferred to cold storage until time of issue. Before dispatch each bottle is individually examined, and any showing an unusual degree of cloudiness or sediment are

rejected. As a control test in purity, agar, and broth cultures are also made from sample bottles filled from each flask.

So prepared and controlled the vaccine is then supplied for use, labelled with the date before which it must be used. This date is taken as a month from the time of issue from cold storage in the case of the "first lymph" of the double vaccine, and up to six weeks in the case of the "second lymph." According to present recommendations the use of "double" vaccine is advised for all classes of stock—in the dose of $\frac{1}{2}$ c.c. per head at each inoculation for cattle, and of $\frac{1}{4}$ c.c. per head for horses, mules, donkeys, sheep, and goats. The dose of the "single" vaccine prepared for the inoculation of cattle only, is $\frac{1}{2}$ c.c. for each animal.

In practice vaccination is largely undertaken by stock owners themselves, but it is also carried out extensively by the Government veterinary officers, or the inspectors of stock serving under them, especially in those cases where it has to be enforced as a measure of State control. In the instructions issued with the vaccine, its use on clean farms is not advised except in those cases where the local conditions are such as to render it extremely probable that infection may be introduced from sources of infection existing in the immediate neighbourhood. In actual practice, however, its use is only resorted to in the majority of cases when infection has already made its appearance, and then usually only after a number of animals have actually died from Anthrax. Even under such conditions a cessation of mortality frequently occurs within a few weeks after the vaccination has been completed. In some instances it is true the number of deaths from Anthrax increases immediately after vaccination, and it has also been noted that at times mortality continues to occur beyond the period of fourteen days from the time of completion of vaccination—within which immunity is usually accepted as being conferred—and even up to the fourth week from this time. This, however, refers more particularly to those cases where the animals vaccinated have been left to graze over the area on which infection was originally acquired. Under such conditions adverse results might be expected—the negative phase following vaccination most probably accounting for the more immediate increase in mortality by favouring the development of infection in those animals which had already acquired it, and the more remote deaths being accounted for by continued exposure to infection before immunity has had time wherein to become established. Whether this be the correct explanation or not, practical experience indicates that the best results from vaccination are to be expected when the animals are removed immediately after inoculation from the infected area to a clean or less-infected one, and there kept for at least a month before return to the former. Considering that beneficial results have been obtained in a large number of cases, there is therefore good reason for advocating the practice of vaccination, even though definite judgment as to its exact value be suspended until more extended experience has been acquired, and more complete statistical information is perhaps available. At the present time, one must be mainly guided by the general opinion of veterinarians and others concerned with the application of the method on a large scale in the field. If provisional evidence of this kind be admitted as justifying a conclusion, there is ground for the belief that in the majority of cases, vaccination has yielded results of a satisfactory character, and that in general its efficacy has been at least as great

as the favourable records from other countries allowed us to expect. But, while the general results may be said to be satisfactory, complaints have been received at times in some cases concerning accidents apparently resulting from the vaccination; in other cases concerning failure to produce the expected immunity. The complaints received, however, have been extremely few in comparison with the number of doses issued, and it is practically certain that, if the losses due to vaccination could be expressed statistically, they would sink into insignificance in comparison with the main beneficial results. But although accidents and failures are rare, their importance as a source of dissatisfaction is great because of the way in which the incidence of loss or inconvenience entailed through them is distributed; an incidence not dispersed in a general way, so as to affect a number of owners in a slight degree, but usually borne by a few individuals. The vaccine-maker is mainly concerned because the occurrences complained of are not recognized by the users of the vaccine as dependent upon any possible inherent defect in the Pasteurian method itself, but are invariably attributed to faulty preparation of the vaccine supplied. The statistical minority of unfortunate accidents is therefore of sufficient importance to those concerned as to justify our devoting the remaining portion of this article to their consideration. The unfavourable aspect of vaccination which they serve to bring into prominence, and the probabilities underlying their causation, may best be discussed separately under the headings of:—

- (1) Post-vaccination accidents; (2) failures of vaccination to produce immunity.

Post-Vaccination Accidents.—That accidents are not more common than they actually are might be remarked upon as a rather surprising fact in view of the number of known cases in which the vaccine has not been administered in accordance with the instructions supplied for its use, but with varying degrees of disregard for them.

Although maladministration is a side issue and need not be dealt with in discussing post-vaccination accidents proper, it may be of interest to cite a few instances brought to our notice, such, for instance, as the use of unsterilized syringes or of unclean receptacles into which the vaccine is poured at the time of the operation; the mixing together of “first” and “second” vaccines for injection as a “single” (!) vaccine into animals not previously inoculated; the injection of a dose much larger than that recommended, or of the second vaccine before the first; the removal of some of the vaccine from a bottle and the keeping over night of the remainder for use next day; the once recorded injection from the nozzle of the syringe into a puncture in the skin made with a nail, because of the loss of the syringe needle; and, finally, an apparently common practice of abstracting some of the material from the bottle by pushing the needle through the cork and keeping the remainder for use several days later, when the effects of that first injected have been observed and passed as satisfactory. Curiously enough, however, disaster appears to have seldom followed in the wake of such extreme misuses of the vaccine, and it is not to conditions arising out of them that we have occasion to refer. Our present concern is with undesirable sequelae in cases where the injection has been properly carried out. The character of

such sequelae has varied. In the majority of instances the development of swellings on different parts of the body—not necessarily present at or developing from the actual inoculation site—has been observed. In other cases there has been noticed a lameness or stiffness in movement, affecting one or more of the limbs but not associated with swellings. In a small minority of cases mortality has occurred.

By way of illustration, the following records may be taken in detail:—

Farm 1.—Vaccine used, single (L); dose $\frac{1}{4}$ c.c.; animals affected, bovines. Anthrax not previously existent on area where animals were kept or were working. 134 working oxen inoculated behind the shoulder, allowed to rest for about 48 hours after the inoculation, and then again put to usual transport work. From about the seventh day after vaccination oedematous swellings were noted in a large proportion of the animals, and by the ninth day were apparent in practically all members of the troop. These swellings are stated to have been absent at the inoculation site, but present in the lower part of the dewlap and chest, extending in some cases backwards along the lower aspect of the abdomen to the sheath. In some cases the foreleg on the side on which the inoculation was performed was also swollen. In 33 animals the swellings developed to such a marked extent as to interfere with movement. After about ten to fourteen days from the time of their first appearance they had again disappeared. During the time they were present the animals fed well, and apart from the associated mechanical interference with movement no other symptoms were noted. Only one of the animals thus affected died, death taking place on the thirteenth day after inoculation.

Farm 2.—On Farm 2 (a native location) an outbreak of Anthrax occurred in December, 1916, and it is estimated that about 200 bovines died before vaccination was carried out in January, 1917.

In at least the earlier period of the outbreak the conditions were such as might be expected to have produced a widespread dissemination of infection over the grazing area and a relatively heavy infection of some parts of the location. A large number of carcasses had been cut up and consumed by native owners, about eighteen of whom themselves contracted the cutaneous form of the disease. In the third week of January, 1917, the survivors of the herd, numbering about 6700, were inoculated with single vaccine (L) in the dose of $\frac{1}{4}$ c.c. per animal. Deaths from Anthrax continued to occur in considerable numbers for several weeks after inoculation, and up to the sixth week about 211 animals are reported to have died. By about the ninth week, however, the mortality, which had been progressively decreasing from the time of inoculation, ultimately ceased. Of the 6700 cattle inoculated swellings were observed in twenty-two animals, and of these eight died. The earliest time after inoculation at which these swellings were noted was about the ninth or tenth day, although in some cases they did not appear until the third or fourth week; and while in some instances they disappeared in about seven to ten days, in others they persisted for some weeks. In the majority of cases they were located in the lower portion of the dewlap, the front of the shoulder, and the forearm of the same side of the body as that on which the inoculation was made, but in four animals they were present in the posterior portion of the body, extending to the hind limbs in two. In one of these four individuals the swelling developed in the posterior portion of the lower aspect of the abdominal wall, and thence

extended to the upper parts of the inner faces of both thighs. In this case the appearance of swelling was noted three days before death, which occurred twenty-eight days after inoculation. In two others the posterior fourth of the abdominal wall, lower portions of both flanks and precrural regions were involved, while in the fourth animal there was oedema of the perinaeal region and also in the upper part of the inner faces of both thighs. These swellings were not painful, and even the animals in which they persisted for some time did not appear otherwise visibly sick, but continued to move about and feed as usual. In six other cattle stiffness and difficulty in movement occurred. In five of these the symptoms appeared about three to four weeks from the time of vaccination, and in the sixth animal in the fifth to sixth week from this time. Once present they persisted for several weeks, except in the case of one animal in which they were noted only three days before death, a month after the inoculation. In three animals stiffness was present in all four limbs; in a fourth in both fore limbs; in a fifth in the left fore and both hind limbs. In a sixth, which also showed a swelling of the left forearm, first the right fore and later both hind limbs, were affected.

Farm 3.—On the 19th January, 1917, a transport ox belonging to the owner of Farm 3 died on the road. Anthrax not being suspected as the cause of death, the carcass was placed upon a wagon, brought back to the farm, and there cut open. Blood-smears brought to this Laboratory on the same afternoon showed Anthrax. The carcass was therefore buried, and the animals on the farm, consisting of seventy cattle and two horses, were inoculated on the evening of the same day with a $\frac{1}{4}$ c.c. of single vaccine (L) and kept from work for about three weeks thereafter. On the tenth day after inoculation one ox developed a swelling of the right fore limb, from the elbow almost to the fetlock, very marked in the region of the forearm. Seen on the twelfth day the swelling was very pronounced, but less so than it had been two days previously. The animal was still able to walk about and graze. The temperature was 101.6° F., and blood-smear examination negative. In about a week from the time it was first noted, the swelling disappeared. Between the tenth and sixteenth days two other animals showed slight swellings in the neighbourhood of the inoculation site, but these disappeared within a few days. Similar swellings were noted in two more cases between the sixteenth and twenty-second days, and in a sixth between the twenty-second and twenty-ninth days. One ox died from Anthrax on the nineteenth day. A seventh animal developed a swelling on the twenty-fourth day, but did not, however, come under our own observation until ten days later. Development was stated to have commenced in the prescapular region, extending to the lower part of the dewlap and the right foreleg in the course of a day or two. When the case was seen on the thirty-fourth day after inoculation there was a marked swelling of the whole dewlap, and the right fore limb was swollen from the elbow to below the knee, especially the forearm. The animal had continued to move about and feed as usual from the time the swelling was first noted, and at the time of inspection its appearance was bright. The temperature was then 105.6° F. On the same day, 22nd February, this ox was transported to the Laboratory and there kept under observation until the 10th March, by which time the swelling had completely disappeared from the dewlap and the limb. On admission the forearm was

punctured with a hypodermic needle and the liquid thus obtained was examined microscopically and culturally. Rare bacilli morphologically resembling Anthrax were present in Giemsa and Methylene-blue stained preparations, some containing small refractile points of different size in their interior, and some "ghost" forms were also present. From this examination it appeared that these organisms represented Anthrax bacilli which had undergone degenerative changes. Culturally no growth whatever was obtained on agar and in broth heavily inoculated. Eight mice and three guinea-pigs were injected—the former receiving $\frac{1}{4}$ c.c. each subcutaneously, and the latter 2.2 and 4.5 c.c. respectively—with negative results. On the day following admission the swelling commenced to decrease in size. The animal was bright and feeding well. Temperatures morning and evening were 103.2° F. and 104° F. respectively. From this time onwards the swelling continued to decrease, and the temperature returned to a practically normal level, with the exception of evening records of 103° and 103.2° on the 25th and 27th February respectively.

The animal continued to feed well and to appear bright, and on the 27th painting of the skin over the swollen region with iodine-tincture was instituted, the leg and dewlap being thus treated on alternate days for a week.

The swelling steadily subsided, first from the dewlap and later from the leg, until by the 7th March it had completely disappeared.

Farm 4.—Twenty head of cattle running on Farm 4, on or in the neighbourhood of which Anthrax had not been existent, were inoculated behind the shoulder with $\frac{1}{4}$ c.c. of single vaccine (L) on 26th January, 1917. On the 1st February it was noted by the owner that in one animal the forearm of the limb of the inoculated side was swollen. The farm happened to be near the Laboratory and the animal was procured for observation. The notes given here refer to this case:—2nd February: At time of admission forearm markedly swollen from immediately below elbow-joint to carpus. Less marked swelling between carpus and fetlock. Slight swelling of the lower part of dewlap, extending posteriorly to between the fore-legs. Animal walked $1\frac{1}{2}$ mile to Laboratory from farm without difficulty, feeding and ruminating as usual. Appears bright. Temperature 106.8° F. Blood-smear negative. On palpation of swelling of dewlap, and upper forearm, fluctuating character evident, and contents appear to be in great part liquid. Forearm punctured with hypodermic needle and the clear slightly yellow liquid, flowing freely from the needle, collected and examined. Microscopically, Giemsa and Methylene-blue staining revealed rare but typical Anthrax bacilli. Cultures made from the liquid on agar slopes and ordinary broth showed a well-developed pure growth in all tubes in twenty hours. Four guinea-pigs and four rabbits were injected subcutaneously from the broth cultures, in order to determine the virulence of the strain. Four guinea-pigs all died within the usual period within which the single vaccine produces death when tested for virulence, while the four rabbits survived. The organism isolated therefore appeared to possess the same degree of virulence as that present in the single vaccine originally injected into the animal on the 26th January. 3rd February: Temperature, M. 104.2° F., E. 105° F. The swelling appears to have increased below the knee. Animal appears bright and is able to rise without assistance. From this day onward the leg

was fomented daily with a warm 5 per cent. carbolic acid solution. 4th February: Temperatures, M. 101.8° F., E. 102.6° F. Appears bright and feeds well. Blood-smear examination negative. 5th, 6th, 7th, and 8th February: Temperatures taken once daily on these dates were respectively 104.6° , 104.4° , 102.8° , and 102.2° F. Swelling about the same. Animal bright and feeding. 9th and 10th February: On the 9th the swelling of the dewlap commenced to decrease in size, and this decrease appeared more marked on the 10th. Temperature on evening of the 10th, 102° F. 11th February: Temperature, M. 101° F., E. 102.2° F. Swelling of dewlap still decreasing. 12th February: Temperature, M. 101.2° F., E. 102.2° F. Swelling in dewlap has almost completely disappeared, and that of leg commences to decrease in size. 13th February: Temperature, M. 100° F., E. 102.6° F. The temperatures on the 14th, 15th, and 16th February were, on the mornings and evenings of each of these days, 101° F., 102° F., 100.6° F., 103° F., 102° F., and 102.4° F., respectively. On the 16th the swelling below the knee had completely disappeared, by which time the swelling had also practically gone from the forearm. The animal was discharged on the 17th February. In this animal the swelling therefore appeared about the fifth, and remained up to about the twenty-second day after the inoculation. The strain of Anthrax organism recovered from the swelling corresponded in virulence to that used for the preparation of the single vaccine, so far as could be determined from the small-animal inoculation tests. It is also to be noted that the animal had been running on veld not known to be infected prior to or since the performance of vaccination.

Farm 5.—On this farm, tenanted solely by natives, nine cattle had died from Anthrax before vaccination was carried out. These deaths took place in January, 1917, two on the 21st, one each on the 22nd and 24th, and five on the 25th of that month. The carcasses of the first two had been opened and part of one eaten before the arrival of the police. Portions of the other were being dried as "biltong," but were buried on police instructions. On 26th January the remaining 270 head were inoculated each with $\frac{1}{2}$ c.c. of single vaccine (L) under the direct supervision of the writer. One ox appearing sick on this day—temperature, 102.4° F.—was not inoculated until all the others had been injected. It had recovered on the 30th January when the farm was next visited. Twenty deaths occurred from Anthrax subsequent to the injection, as follows:—Two on the 2nd day, three on the 3rd day, three on the 4th day, four on the 6th day, two on the 7th day, one on the 9th to 10th day, one on the 10th to 11th day, one on the 18th day, one on the 40th day, one on the 41st day, one on the 49th day. On the 45th day after the inoculation, the survivors were reinoculated, 99 with single vaccine (I) and 151 with single vaccine (L), all with a $\frac{3}{4}$ c.c. dose on this occasion. Four days later another death from Anthrax occurred, but thereafter no further mortality was observed. On 30th January one group of eight cattle and on 31st January another group of the same number were noted to be sick and not feeding well. In the majority of cases the temperature was elevated, in some considerably so. Of these sixteen animals ten recovered and six died. The following notes relate to these cases,

the days in the tables being reckoned from the time of the first inoculation:—

Animal.	Noted Sick.	Died Anthrax.	Temperature and Symptoms, 4th Day.	Temperature and Symptoms, 5th Day.
1st Group:				
1	4th day	6th day	107.8°	Respiration snoring. Amaurosis. Blood-smear = 0.
2	2nd day	4th day	103° Bleeding from wound on ear produced by ticks. <i>B. anthracis</i> extremely numerous in this blood. Died half an hour afterwards	—
3	4th day	—	106.4°	Appeared better.
4	4th day	—	107°	107° Blood-smear = 0.
5	1st day	—	102.4°	Appears better.
6	1st day	6th day	106.8° Respirations increased. Nose very swollen	104.8° Head swollen and resembles that of a hippopotamus. Blood-smear = 0.
7	1st day	6th day	107°	107° Blood-smear = 0.
8	4th day	4th day	106°	—
2nd Group:				
9	5th day	—	103° Respirations increased. Blood-smear = 0	—
10	5th day	—	103.6°	—
11	5th day	—	107° Small swelling on right side dewlap. Blood-smear = 0	—
12	5th day	—	103.8°	—
13	5th day	—	104.2°	—
14	5th day	—	106.8° Blood-smear = 0	—
15	5th day	—	102.4°	—
16	5th day	6th day	107.8° Blood-smear = 0	—

In all, nine cattle out of the 270 showed swellings which appeared on different dates. In three of these, including No. 11 in the above table, they appeared on the fifth day after inoculation. In one (No. 11) the swelling was situated on the right side of the lower part of the dewlap; in another it was present on the upper border of the neck in its posterior third and about the size of two fists. In the third the site of inoculation was swollen. In a fourth case the left foreleg and front of the shoulder were swollen from the sixth day after inoculation. When seen on the ninth day the temperature of this animal was 105.4°. Blood-smear examination negative. Four others showed swellings appearing between the seventh and ninth days at the inoculation site. The temperature of the one in which the swelling was largest was 105.8° on the ninth day. Blood-smear examination negative. In the ninth animal the lower part of the dewlap was

very swollen from the twelfth day. On the sixteenth day the temperature was 107.6° and blood-smear examination negative. On the eighteenth day this animal died from Anthrax. In the other cases the swellings had disappeared by about the sixteenth day, with the exception of the fourth, in which the swelling of the foreleg remained a few days longer.

Apart from the cases just detailed with special reference to cattle, a few instances of post-vaccination accidents may be recorded in which equines and goats were affected.

One of these refers to a farm on which vaccination was carried out because of the death of a bovine from Anthrax shortly before. Several hundred bovines and fifty equines were inoculated with $\frac{1}{4}$ c.c. of single vaccine (L).

Twenty-six of these equines were geldings and none of these showed untoward symptoms, but of twenty-four mares, injected with the same material and using the same syringe immediately after the geldings, quite a number showed more or less extensive swellings. One of the mares, inoculated in the neck after due disinfection of the inoculation site, was described by the owner as much swollen on the neck and shoulder a day later and markedly lame. Her respiration was quickened and appearance distressed. On the day after the swelling had decreased in size in the shoulder region, but now involved the head, which on the third day was very much enlarged. Puncture of the skin allowed the escape of a yellowish serous fluid. On the fourth day she appeared slightly better, but the lips were now somewhat more swollen than before. During all this time she did not feed, and death occurred a few days later. In two other mares, said to have appeared very near to death before recovery eventually took place, the swellings remained for about a week before finally disappearing. The owner mentions that at the time of inoculation all the mares were "very low in condition and had foals at foot."

In another case, which was reported by a veterinary officer, two mares died fifteen and thirteen days respectively after inoculation of single vaccine (L), $\frac{1}{4}$ c.c. dose. In the first of these, swellings containing an amber-coloured gelatinous material were observed post-mortem. One was situated on the near side of the trunk; another, measuring 20×18 inches, on the under aspect of the thorax; and a third, of considerable size, was present on the inside of the near thigh. No swelling was noted in either case at the site of inoculation. Post-mortem decomposition did not allow of complete examination of the second animal.

A final case may be described, in which a number of goats were inoculated with single vaccine (L), $\frac{1}{8}$ c.c. dose. Within eight days between 40 and 50 died. Before death a number showed swelling of the inoculated limb, extending down to the hock, and underneath the abdomen and thorax. In six blood-smears out of twelve submitted from dead animals Anthrax bacilli were found.

The preceding records will suffice to illustrate the types of post-vaccination accidents encountered, and to show that in character they are similar to those which have been associated with the application of the Pasteurian system in many other parts of the world. Although long and well recognized, their origin, nevertheless, remains somewhat obscure. Many views have been put forward at different times in the

literature on Anthrax vaccination, some of which we may quote later in further discussing our South African experience. Dealing in a general way with accidents following anti-Anthrax inoculation, Hutyra and Marek, under the heading "Direct Results from the Vaccination," remark:—"Fatal results may sometimes follow, even when the vaccination is carried out correctly with faultless vaccine, probably as a result of the low individual resistance of the animal. In several instances of this kind numerous losses were observed."

To the occurrence of post-vaccinal accidents in France in connection with inoculation against both Anthrax and Swine-erysipelas, Chamberland has referred in 1894, offering the explanation which we here quote in citing from an article by McFadyean (²⁷), published in the same year. This article deals with certain unsatisfactory trials of the Pasteurian method in Great Britain, and in the course of which a considerable mortality has been noted amongst a low of ewes following the injection of the second vaccine. The passage cited reads:—

"It must be admitted that the result of these trials of the Pasteurian method of vaccinating against Anthrax is very unsatisfactory. It may be, and no doubt is, true that when thousands of animals are vaccinated the percentage of loss from the operation itself is a mere fraction per cent., but here we have a case in which in one lot of ewes 7 per cent. of the animals succumbed to the operation intended to protect them. If this had been the first recorded instance of the kind one might have supposed that the unfortunate results were accidents in the strict sense of the word, that is to say, ascribable to some imperfection or mismanagement in the method of carrying out the operation. But, unfortunately, it appears that accidents of precisely the same kind occur every now and again in France, and that no human foresight can prevent them. Thus, in a note to the most recently published statistics regarding the Anthrax vaccinations in France, M. Chamberland admits that accidents of this kind occur here and there every year after the vaccinations. He says that while ten, fifteen, or twenty veterinary surgeons receive on the same day the same vaccine and carry out the vaccinations without accident, it sometimes happens that one of them reports that a few days after the vaccinations 5 or even 10 per cent. of the animals have succumbed to Anthrax. These accidents are all included in the statistics, but they are so rare that they hardly influence the final result. They are deplored, because they raise a serious prejudice against the system of vaccination when they become known, and it is admitted that they have always been a great puzzle to those who are responsible for the manufacture of the vaccine. Now, however, M. Chamberland thinks that he has discovered an explanation of them, which he gives in the following words:—"In the first place, almost all these accidents take place after the first vaccination, and that leads us to think that very often the animals succumb not to the inoculation, but to the spontaneous disease which already existed in the animals, and which was just on the point of manifesting itself. Sometimes, it is true, animals die after the second vaccination, or even after the first, with symptoms which seem to indicate that the disease had its starting point at the seat of vaccination. The vaccine itself cannot be incriminated, since the same sample is sent on the same day to other veterinary surgeons, in whose hands it does not produce any ill effects. It is probable that the breed of the animals or the mode of feeding may play a certain rôle, but that cannot be important since the accidents occur everywhere in every corner of France.

"We think rather that they ought to be attributed to some accidental impurities which have been introduced under the skin at the same time as the vaccine. In fact, we know to-day beyond any doubt that two microbes which when inoculated separately under the skin of an animal do not produce any injurious effect, may, when they are associated, entail a fatal result. But when one reflects on the conditions in which the inoculations are ordinarily performed—in buildings, on animals having the skin soiled with dirt, with syringes the needles of which are bound to be contaminated—one is bound to admit that impurities must be frequently inoculated at the same time as the vaccine. Hence these purulent oedemas which have been reported to us. We think that the presence of foreign microbes is the principal cause of the accidents in question. It does not appear to us to be possible to avoid them altogether, for in the

practice of the operation on a large scale one cannot employ the precautions which are customary in laboratories. But they may be avoided in part by remembering that every impurity introduced under the skin at the same time as the vaccine may entail fatal consequences.”

An explanation, different, however, from Chamberland's, is that advanced by Leclainche and Vallée, in an article on “The Pathogenesis and Prophylaxis of the Accidents following Protective Inoculation” (28), in which it is stated that:—

“All the methods of immunization by means of attenuated virus are liable to accidents, and although the general percentage of these is not at all great they cannot be altogether neglected, and when they occur they may be the cause of considerable loss to individual owners.

“The same facts are observed in the case of the three diseases against which it is possible to vaccinate by using a modified virus, viz.: Anthrax, Swine-erysipelas, and Quarter-evil.

“Vaccination against Anthrax is undoubtedly the most certain in its results, but, at the same time, one now and again hears that it has caused a certain mortality in the hands of some experienced veterinary surgeon. In an exceedingly interesting article, Bigoteau has published some striking examples of these annoying surprises. Similar facts have been observed in every Anthrax country.”

This actual explanation appears in the following extract:—

“Except in very rare circumstances it is impossible to explain the accidents by assuming that at the moment of inoculation the disease had already begun to develop. It is puerile to invoke such coincidence when half of the vaccinated animals are affected at the same time after the operation. The relation between the accident and the operation is evident. ‘It is not the vaccine, but the vaccination that kills.’

“One conclusion that must be drawn is that the accidents are due to variations in the susceptibility of the animals vaccinated. In order to explain this all the ordinary factors of etiology have been invoked, and when it became manifest that they were insufficient one fell back on unverifiable hypotheses. In reality the accidents are *almost always* due to a *latent infection* with the virus against which one is endeavouring to protect the animal. That is the occasion which permits the bacterial invasion and evolution.”

McFadyean (27-29), however, criticizes both of these views. In regard to Chamberland's, he remarks that, while admitting that in certain cases the introduction of vaccines contaminated with extraneous organisms may be responsible for the death of some animals, there is no evidence or probability in the view that this happens at all commonly. According to him, “in the immense majority of cases the so-called ‘inoculation Anthrax’ and ‘inoculation Quarter-evil’ is a pure infection in which no second organism plays any part.”

Leclainche and Vallée's explanation he criticizes chiefly on the evidence gained from his own observations in regard to the incidence of Quarter-evil, and the result of inoculation against it in Great Britain. Summing up this evidence, he finally states his opinion that “. . . the latest infection theory of the accidents that follow vaccination against Quarter-evil must be pronounced unsatisfactory and improbable, and any attempt to apply the theory to Anthrax and Swine-erysipelas is open to similar objections. The explanation that still appears to be most in harmony with the observed facts is that individual susceptibility—especially with regard to Quarter-evil—varies within rather wide limits, and that a vaccine that has been found perfectly safe for a large number of animals may prove to be too strong for the individuals whose powers of resistance are much below the average.”

From Madagascar, Grandmougin (12) records accidents following the practice of the classical Pasteurian method by Carougeau in

Tananarivo district, and also after the use of the Chauveauian vaccine. He also is inclined to regard them as chiefly dependent on differences in individual susceptibility of the vaccinated animals.

The citations may be taken as representing authoritative opinion concerning the origin of post-vaccination accidents, and it only remains to add some comment based on the experience derived in this country. In reviewing the cases we have already taken for illustration, it is not considered that the accidents are at all to be explained on the lines suggested by Chamberland, since they have been chosen as examples of instances where the vaccine was inoculated with all due care and with full observance of the conditions of cleanliness required. On one of these farms (Farm 5) the inoculation was carried out under the writer's direct supervision. On three others (Farms 2, 3, and 4) by lay officers of the veterinary service, and on Farm 1 by an individual possessing considerable previous experience of the operation. That they are to be accounted for by the existence of a latent infection, in the sense of Leclainche and Vallée, is an explanation also difficult to accept. The possible existence of such an infection might be admitted for those animals on the farms where Anthrax was known to be present, but in two cases (Farms 1 and 4) there is no reason to suspect this. The history of both cases showed that no deaths from Anthrax had occurred in the herds before inoculation, and that the animals were not exposed to infection after the operation. Yet, on one farm, 33 out of 134 animals developed swellings and one died. On the other only one animal showed a swelling, and from this it was possible to recover a strain of organism corresponding in virulence possessed to the attenuated strain of bacillus used in preparing the vaccine injected.

In regard to the possible influence of the vaccine strains upon the incidence of the post-vaccinal accidents, it may be recalled that in all our cases the single (L) variety was injected in $\frac{1}{4}$ c.c. dose for bovines ($\frac{1}{2}$ c.c. one case), $\frac{1}{4}$ c.c. for equines, and $\frac{1}{8}$ c.c. dose for small stock. Since this strain possesses a degree of virulence closer to the second of the two vaccines used in the double method, it might be held by some as to be in a certain measure responsible for the results. Such explanation is, perhaps, admissible where animals such as equines, sheep, or goats are concerned, but is less possible in bovines, and it is to be remembered that similar accidents have been recorded in other countries as following the use of the classical double method. To infection by subcutaneous inoculation, even with fully virulent cultures, bovines are known to be very resistant. Oemler is quoted by Nocard and Leclainche as having only succeeded in producing death by this means in one out of forty-one animals, and it is, therefore, not surprising that cattle stand injection with single vaccine in doses much larger than usually employed without outwardly apparent effect. The absence of external symptoms after a dose of 1 c.c. instead of the usual $\frac{1}{4}$ c.c. of the single vaccine has been noted in two field experiments in which Viljoen and Robinson of this Division injected 76 and 75 head of cattle, respectively, never previously vaccinated. While discussing here the effects of the single vaccine, it may also be well to note that in preliminary tests made on some hundreds of cattle, $\frac{1}{2}$ c.c. appeared to be borne so well that it is now adopted as the dose in general use in the field wherever single vaccination is still practised.

Moreover, this manifestation of resistance is not confined only to

injection of the single vaccine. In one test made by the writer, fifty-five cattle, not before inoculated, were directly injected without any externally visible result, with $\frac{1}{2}$ c.c. each of the second culture of the double vaccine. Since then it has been noted that Grandmougin, in view of Oemler's experience, also came to consider that the second vaccine might be injected direct—thus saving the inconvenience of performing two inoculations on native-owned cattle—and, being satisfied with preliminary tests, subsequently carried out inoculations of some thousands of cattle in Madagascar, using one direct injection of $\frac{1}{4}$ c.c. of the imported Pasteur second vaccine.

In view of all this, then, it may well be asked with Leclainche and Vallée: "How, then, can one explain the fact that one-quarter cubic centimetre of a virus so weak as the first vaccine, which will hardly kill a rabbit, is liable to set up in certain individuals of the bovine species a virulent and fatal attack?" That two factors suggested as causing the accidents—injury and work—may play a certain rôle in their production may be allowed for in some, although not in all, of our cases. On Farm 1 the animals were put to work forty-eight hours after vaccination, but on Farms 2, 3, 4, and 5 they were simply left to graze over the veld. On the whole, however, McFadyean's view that all the post-vaccination accidents are ultimately dependent upon the possession by the animals affected of a degree of individual resistance below the average, seems to offer the most feasible explanation for their occurrence. But whatever may be the final conclusion as to their causation, a point of practical importance, confirmed by the experience in this country, is that the risk of encountering them is much increased by the use of the single vaccine. On this account the issue of this type of vaccine for the injection of equines, sheep, and goats, was completely discontinued in March, 1917, and restrictions were placed upon its use for cattle at the same time. Since then a large amount of the double vaccine has been issued, and the results with this would certainly appear more satisfactory so far as the risk of accident-production is concerned. During the last fifteen months only three cases of post-vaccination accidents have been reported. One of these relates to the occurrence of mortality amongst sheep, and the second and third to the appearance of swellings at the inoculation site in a very few cattle and donkeys respectively. The first of these cases, however, could also be accounted for through very probable natural infection existing at the time of, or developed shortly after, the vaccination, while the other two, even if not explainable except as directly due to the inoculation, are not regarded as occurrences of character sufficiently serious to cause alarm.

Failures of Vaccination to Produce Immunity.—In proof of the general benefits of Pasteurian vaccination extensive statistics collected in a number of countries where it has been applied on a large scale have been published in different places. The evidence they furnish on the point is summarized by Hutyra and Marek (*l.c.*) as follows:—

"Up to the end of the year 1899, 708,980 cattle and 4,971,494 sheep had been vaccinated successfully in France, as a result of which the entire annual loss over a period of twelve years dropped to an average of 0.34 per cent. in cattle and to 0.94 per cent. in sheep. In Hungary the first vaccinations were carried out in 1881 on Azary's initiative. Thuillier, Pasteur's assistant, applied them first at the Veterinary Institute at Budapest with very favourable results, while the later ones in Kapuvár were less favourable. As vaccination gave very good results in practice, this protective treatment was soon taken up very

extensively. During the period of fifteen years between 1886 and 1900, 53,843 horses, 1,015,700 cattle, and 2,279,221 sheep were vaccinated. The official report contains the results on 39,506 horses, 718,266 cattle, and 1,247,331 sheep, and according to the statements the percentage of vaccinated animals given below died of Anthrax:—

	Horses.	Cattle.	Sheep.
	Per cent.	Per cent.	Per cent.
After the first vaccination.....	0·10	0·02	0·26
In the course of following years.....	0·09	0·02	0·33
TOTAL.....	0·19	0·04	0·59

“In Germany the results were at first less favourable. (In Pockish during the period 1882 to 1888 the entire loss of vaccinated cattle amounted to 3.1 per cent. and of vaccinated sheep 2.8 per cent.) Later, particularly in Württemberg and Alsace Lorraine, the results were more satisfactory. . . .

“In Austria vaccination was carried out on 9456 fattening cattle with complete success during the period between 1894 and 1898. It was observed, however, that the vaccination of such animals during the summer months caused a severe reaction.

“In Russia vaccination has also been followed by uniformly good results, with the exception of the fatal vaccination in the Crimea (1888) which was supposed to be due to an error in the vaccines, in which instance 4564 sheep were vaccinated and 3478 died in the course of a few days. The total loss in the District of Cherson, in which 20,000 sheep were vaccinated during the years 1885-1888, amounted to 0.87 per cent. compared with previous losses of 10 per cent. to 33 per cent. in former years (Wysokowicz).

“Similar good results followed vaccination in Holland (Wirtz), in Switzerland (Hess), as well as in America (Dalrymple), and Australia.”

More recently compiled statistics are those of Lukacs. ⁽²⁰⁾

Lukacs states that the system of cultural vaccination has been practised according to regulations for more than twenty-five years in Hungary, and that he has succeeded now in finding a most efficient protection against Anthrax without serious complications. The statistics regarding the vaccination of animals in healthy and infected herds furnished by him show that (1) of 8,150,650 healthy animals vaccinated there were after vaccination 4493 dead, viz., $\frac{1}{2}$ per mille; within a year, in spite of vaccination, there were 10,951 deaths, viz., $\frac{1}{3}$ per mille. (2) Of 411,654 animals exposed to infection before vaccination, hence suspected of infection, there were 1493 dead, viz., $\frac{1}{3}$ per cent., and, in spite of vaccination, within a year 3050, viz., $\frac{3}{4}$ per cent. In the summarized table furnished by Lukacs the actual numbers of each species inoculated and other details being concisely given, these are reproduced below:—

HEALTHY ANIMALS INOCULATED.

SPECIES OF ANIMAL.	NUMBER.	DEATHS.			
		Between 1st and 2nd Inoculation.	Per cent.	In Spite of Vaccination.	Per cent.
Sheep.....	4,034,432	4,074	0·1	9,177	0·25
Cattle.....	3,920,350	369	0·01	1,432	0·04
Horses.....	195,861	50	0·02	142	0·06
TOTAL.....	8,150,643	4,493	0·05	10,751	0·13

ANIMALS INOCULATED IN HERDS EXPOSED TO INFECTION AT TIME OF INOCULATION.

SPECIES OF ANIMAL.	NUMBER.	DEATHS.			
		Between 1st and 2nd Inoculation.	Per cent.	In Spite of Vaccination.	Per cent.
Cattle.....	241,526	517	0·2	1,182	0·5
Sheep.....	167,448	963	0·6	1,849	1·1
Horses.....	2,680	13	0·5	19	0·75
TOTAL.....	411,654	1,493	0·3	3,050	0·75

Of the value of vaccination in American experience Dalrymple ⁽²⁰⁾ writes:—

“In these parts of the United States of America, and more especially in the semi-tropical latitudes which perhaps have suffered most from this disease in the past, live stock sanitary legislation has now been enacted; greater areas of land are being rapidly placed under cultivation and drainage, etc., with the result that the disease is now being much more intelligently controlled than formerly, and its serious consequences very much lessened. In bringing about these more gratifying results, it is believed that, in addition to more sanitary procedure, protective inoculation has played an important part. It may be said to be the general custom, in those States where infection is known to exist on, or contiguous to, farms and plantations, to vaccinate the live stock sufficiently early each spring to permit of immunity being conferred previous to the warmer season with its heat and moisture, which favour the development of the organism and transmission of the infection to the animals. Where vaccination has been carefully, regularly, and intelligently practised in this way, its salutary effects are often very apparent, as shown in comparison with instances where protective inoculation has not been so used in the same localities.”

As to the length of time for which protection should be conferred. Hutyra and Marek (*l.c.*) state:—

“The immunity reaches the necessary degree in about ten to twelve days after the second inoculation and lasts about one year. (In the experiments of Azary vaccinated sheep resisted the artificial infection even after eight months.) A longer duration of the artificially produced immunity is doubtful, and therefore a repetition of the vaccination is indicated annually in pronounced Anthrax districts. The breed, age, and sex of the animals do not appear to influence the results of the vaccination, and young animals which were only shortly weaned may also be vaccinated without danger and with promising results.”

Nocard and Leclainche write (*l.c.*):—

“The immunity is only complete about fifteen days after the second vaccination; it nearly always suffices to place the animals beyond the risk of accidental infection. The duration of protection varies according to the individual. In troops of animals vaccinated in spring the mortality in the following year reaches on an average about 1 per cent. It can be stated that sheep are still immune to the extent of 60 per cent. at the end of a year (Chamberland). In practice it is necessary to repeat vaccination yearly.”

According to Lukacs (*l.c.*):—

“By cultural vaccination the immunity lasts ten to eleven months, by simultaneous vaccination six to seven months.”

If, then, these statements and a reasonably large experience in this country be taken to indicate that Pasteurian vaccination has generally a high degree of efficacy, and usually confers a protection lasting from at least nine to twelve months, explanation has to be found for those cases in which so-called immunity breakdowns have occurred within the nine-months period. Instances of this kind are given in relating below the experiences on Farms 6, 7, and 8. In a large number of the cases reported there is no doubt that the unsatisfactory results should be attributed to either the use of a non-potent vaccine or improper technique in performing the inoculation. It is known that on occasions the vaccine has been kept for several months or even up to a year before use; and it is also recognized that through clumsy manipulation of the hypodermic syringe, or errors made in attempting to separate inoculated from uninoculated animals at the time of the operation, many individuals escape injection. That all cases, however, are dependent upon one or other or both these possibilities cannot be accepted. Even should it be maintained that the imported vaccine used on Farm 6 may possibly have lost potency, although used in reasonably fresh state, this leaves the other two cases to be accounted for. In neither of these is there any reason to doubt the proper performance of the inoculation, while possible non-potency of the vaccine is considered ruled out through the use of the locally prepared fresh material.

Farm 6.—On Farm 6 two bovines of a herd of about 177 died on 13th October, 1913; Anthrax was diagnosed as the cause of death. The remaining 175 animals were inoculated with $\frac{1}{4}$ c.c. of double vaccine (I), the vaccination being completed by the 7th November. Despite this vaccination, however, deaths still continued to occur, and although a second inoculation with double vaccine (I) was carried out in January, 1914, 41 animals had died up to the 14th March, 1914. Between this date and 8th May three more died; and later in May two deaths occurred, one on the 11th and another on the 14th. On the 12th June, 1914, two deaths were reported; and in July one occurred on the 10th of that month. On the 14th July, 1914, the survivors of the herd were again inoculated; this time with single vaccine (I) in the dose of $\frac{1}{4}$ c.c. Following this injection one death occurred on the 31st July, one on the 11th August, one on the 6th September, one on the 5th November, one on the 13th November, and one on the 30th November. On 3rd December, 1914, fifty animals were injected with $\frac{1}{2}$ c.c. of second vaccine (I) preparatory to injecting the rest of the herd with this dose if the mortality continued, but as no further deaths occurred during the year that followed, inoculation of the remaining animals with the $\frac{1}{2}$ c.c. second vaccine, not being necessary, was not carried out.

Farm 7.—On Farm 7 Anthrax appeared amongst a herd of bovines, numbering about 1100 head, in the latter part of September, 1916, seven animals dying of the disease at that time. On the 1st, 2nd, and 3rd of October the remaining animals were inoculated with $\frac{1}{4}$ c.c. of single vaccine (I), and between this last-mentioned date and the 26th October a further nine deaths occurred. Two of these took place within the first week after the inoculation, four from the tenth to the fourteenth days after, and three within the twenty-first and twenty-third days. A second supply of vaccine

(L) being then obtained, the herd was again inoculated between the 1st and 3rd of November, 1916, that is a month from the time of the first inoculation. On this occasion the dose injected was $\frac{1}{2}$ c.c. per animal. On the sixth day following this vaccination one animal died of Anthrax. There were then no further deaths until the 11th of January, 1917, when two more animals died. In the latter part of the same month another two died, one on the 24th and one on the 26th January. In February, 1917, five deaths occurred: one on the 2nd, one on the 8th, and three between the 19th and 21st. At this time 75 of the herd were reinoculated with a dose of 1 c.c. of single vaccine (L), as it was intended to inject the remaining animals with this increased dose if deaths continued to occur. The mortality ceasing, however, the remainder of the herd did not receive the increased dose. No further deaths from Anthrax have since been reported from this farm. In the latter part of 1917 the herd was again vaccinated, double vaccine (L), injected in the dose of $\frac{1}{2}$ c.c., being used. Amongst the 75 animals inoculated with the 1 c.c. dose of single vaccine no ill effects were noted, except in a few cases in which a slight swelling at the site of inoculation was noted, extending in some animals also to the forearm of the same side. These swellings, however, soon disappeared.

Summary.—In this case, seven deaths had occurred before vaccination with $\frac{1}{4}$ c.c. dose of single vaccine (L) was carried out. Within the first week following this inoculation two animals died. Four more died between the tenth and fourteenth days, and three deaths between the twenty-first and twenty-third days. On the thirtieth day after this inoculation another injection of single vaccine (L) was made with a dose of $\frac{1}{2}$ c.c. Six days later one animal died of Anthrax. Deaths then ceased until the seventieth day from this last inoculation—or one hundred days from the first vaccination—on which day one occurred. One animal died on each of the eighty-third, eighty-fifth, ninety-second and ninety-eighth days subsequent to the second inoculation, and three more died between the one hundred and ninth and one hundred and eleventh days. Seventy-five animals were injected with a 1 c.c. dose of single vaccine (L) a few days after the last-noted deaths occurred.

None of these died, neither did any of the remaining animals which were not inoculated with this larger dose, the mortality ceasing with the deaths recorded on the one hundred and ninth to the one hundred and eleventh days after the second injection. Another vaccination, in which double vaccine (L) in the dose of $\frac{1}{2}$ c.c. per head was given, was carried out in the latter part of 1917 and no deaths from Anthrax have since been reported.

Farm 8.—The information furnished as to the mortality from Anthrax occurring on this farm, during occupation by its present tenant, and the results which followed vaccination, are as follows:—On the 16th December, 1915, a working ox suddenly became ill and died in the yoke, and on microscopic examination of a blood-smear from this animal Anthrax was diagnosed as the cause of death. From the time of this animal's death, mortality from Anthrax continued to occur up to October, 1917, the total number of deaths being 31, including 9 oxen, 8 cows, 6 heifers and tollies, 3 calves, 1 mule, 2

sheep, and 2 pigs. The number of the owner's cattle on the farm at the time the first death took place was 317, made up of 139 oxen, 72 cows, 36 heifers, 7 tollies, 1 bull, 62 calves. In addition there were 200 native-owned cattle on the farm. Anthrax may have been existent on the farm prior to this outbreak, since in previous years a number of deaths of different species of animals had occurred. These, however, had at that time been attributed to causes other than Anthrax. The farm may be described as divided up into a number of parts which we shall refer to respectively as A, B, C, D, and E. All of the owner's cattle were running on the portions A and B; the native-owned cattle grazed on the portion C; and the pigs were styed in a camp, the vicinity of which they did not leave, on portion D. All of the animals which died were watered from a dam situated on portion A, or else drank from kuilen, or waterholes, above or below this dam on portions A and B. It was noted that there were fewest deaths among the animals drinking from the kuilen on portion B, and those of this lot which did die also had access to the water at the dam, or in its immediate vicinity, on A at some or other times. In April, 1916, Veterinary Research Officer Andrews collected from this dam a sample of water mixed with mud stirred up from the edge, and succeeded in proving the presence of Anthrax spores in this material. This dam is believed to have been the principal source of infection. No deaths from Anthrax occurred among a large number of animals watered from boreholes on the portions D and E. The pigs which died in D portion are believed to have contracted the disease from portions of carcasses fed to them before the deaths which occurred in the first part of the outbreak were recognized as due to Anthrax. Once the existence of Anthrax was definitely recognized all animals dying from that time onwards, whatever the cause of death, were buried deeply and intact. Since the dam was finally fenced off at the commencement of December, 1916, there have been very few deaths and these have occurred only amongst animals with access to the kuilen on portion A close to the dam, when the water was very low and muddy. Following the death of the above animal on the 16th December, 1915, one ox died on the 29th of the same month. On the 5th January, 1916, another ox died, and on the 10th January the deaths of a cow and a yearling took place. On 11th and 12th January, all of the owner's cattle were inoculated with single vaccine (I), $\frac{1}{4}$ c.c. dose, and on 11th February all native-owned cattle were inoculated with single vaccine (L), $\frac{1}{4}$ c.c. dose. On the 10th February an uninoculated mule died. Subsequent to these above inoculations a number of others were carried out at different times. Some of these were primary vaccinations of newly introduced animals; others were repeated vaccinations of animals previously inoculated. Some animals received as a final dose even 1 c.c. of vaccine. The deaths which occurred from the 11th February with the number of injections and the quantities and kinds of vaccine which the animals received are as follows:—

Date of Death.	Number and Species of Animal.	Date of Inoculation and Vaccine Used.
1916.		
15th February.....	1 Native-owned ox...	$\frac{1}{4}$ c.c. single (L), Feb. 11th.
19th February.....	1 Cow.....	$\frac{1}{4}$ c.c. single (I), Jan. 11th-12th.
21st February.....	2 Cows.....	$\frac{1}{4}$ c.c. single (I), Jan. 11th-12th.
23rd February.....	1 Bull calf *.....	$\frac{1}{4}$ c.c. single (I), Jan. 11th-12th.
	1 Cow }.....	$\frac{1}{4}$ c.c. single (I), Jan. 11th-12th.
	1 Heifer }.....	$\frac{1}{4}$ c.c. second (L), Feb. 22nd.
24th February.....	1 Cow.....	$\frac{1}{4}$ c.c. single (I), Jan. 11th-12th.
28th February.....	2 Oxen.....	$\frac{1}{4}$ c.c. single (L); Feb. 8th-9th.
9th March.....	1 Merino sheep.....	Not inoculated.
26th March.....	1 Ox.....	$\frac{1}{4}$ c.c. single (L), Feb. 8th-9th.
27th March.....	1 Ox.....	$\frac{1}{2}$ c.c. second (L), Feb. 28th.
		$\frac{1}{4}$ c.c. single (?), a few weeks before arrival on farm.
1st April.....	1 Tollie.....	$\frac{1}{4}$ c.c. single (I), Jan. 11th-12th.
		$\frac{1}{4}$ c.c. second (L), Feb. 24th.
5th April.....	1 Ox.....	$\frac{1}{4}$ c.c. single (I), Jan. 11th-12th.
		$\frac{1}{4}$ c.c. second (L), Feb. 25th.
10th April.....	1 Pig.....	Not inoculated; kept in sty from birth.
		$\frac{1}{4}$ c.c. single (I), Jan. 11th-12th.
8th May.....	1 Tollie.....	$\frac{1}{2}$ c.c. second (L), Feb. 24th.
		1 c.c. second (L), May 5th.
31st August.....	1 Ox.....	$\frac{1}{4}$ c.c. single (I), Jan. 11th-12th.
		$\frac{1}{4}$ c.c. second (L), Feb. 25th.
9th September.....	1 Calf.....	Not inoculated.

On 4th December, 1916, the dam on portion A of the farm was enclosed (see above); and in February, 1917, the stock were reinoculated with double vaccine (L). Deaths since then have been 1 tollie on 12th March, 1917; 1 tollie, 8th May, 1917; 1 calf, 3rd June, 1917; 1 cow, 9th October, 1917; and 1 pig on 19th October, 1917. It is not known how this last animal contracted infection. In March, 1916, the horses and sheep on the farm were inoculated with double vaccine (L), $\frac{1}{4}$ c.c. and $\frac{1}{8}$ c.c. doses respectively. The animals which received a final dose of 1 c.c. second vaccine (L) in 1916 were 20 oxen inoculated on 10th April; 25 yearling cattle inoculated on 18th April; and 41 cattle (38 tollies and heifers, and 3 cows) on 5th May. Of these only one died, death taking place on 8th May, three days after the inoculation. This animal had previously received two inoculations, with $\frac{1}{4}$ c.c. single vaccine (I) and $\frac{1}{2}$ c.c. second vaccine (L), respectively (see above).

The experiences then on at least Farms 7 and 8 are considered to represent genuine failures to produce immunity. To explain them it would seem necessary to postulate the existence of two sets of circumstances, one primarily connected with the vaccinated animal, and the other with its environment after inoculation. The former is probably the less important, but raises two possibilities: (a) That some individual animals are so resistant to vaccination that the reaction called forth is not sufficient to produce a significant

* The bull calf was not inoculated on 22nd February as he was then too ill.

subsequent immunity. It is difficult to estimate the extent to which this assumption is true of species such as equines, sheep, and goats, but with a species such as the bovine, where resistance to subcutaneous infection with even relatively virulent strains is generally recognized, failure to react may perhaps not infrequently occur. (b) That even when the vaccine has definitely conferred a fair degree of immunity infection may still occur under conditions which lower the general physiological resistance of the animal. Amongst conditions operative in this direction may be included anaemia due to helminthiasis or other causes, exposure to excessive heat or cold, or to cold rains, and possibly even dipping. It is not likely, however, that either of these possibilities cover more than a small proportion of observed vaccination failures, and for the majority of cases of ineffective inoculation environmental conditions of infection offer the more plausible explanation. The breakdowns may then be interpreted on a quantitative or qualitative basis, and be regarded as due either to continued exposure to a very heavy natural infection, or perhaps to the existence of peculiarly virulent strains of the Anthrax organism in certain localities. The presence of abnormally heavy infection on a very large number of South African farms must be admitted when one remembers the widespread negligence in regard to the disposal of Anthrax carcasses. It is strikingly illustrated in relation to the water supply of Farm 8. The extent to which differences in degree of post-vaccination immunity can be attributed to differences in degree of pathogenicity of certain strains offers a promising field for experimental inquiry, and investigations in this direction have already been commenced at this Laboratory. On grounds of analogy with other diseases variability of virulence is *a priori* a tempting hypothesis, and in regard to Anthrax it is already recognized that very marked differences in strain-virulence may exist. The contingencies outlined in this section have been kept in view in considering the failures reported in connection with the use of the "L" vaccine, and have served to suggest certain modifications in the application of the Pasteurian system of vaccination in this country. One of the modifications already instituted consists in doubling the ordinary dose of vaccine for all animals, except equines. Since January, 1917, the dose adopted has been $\frac{1}{2}$ c.c. for cattle and $\frac{1}{4}$ c.c. for equines, sheep, and goats. The increase in dosage facilitates quantitative injection, and is regarded as inducing a more vigorous reaction.*

Another modification, recommended in dealing with immunity breakdowns, involves the revaccination of the whole troop or flock, followed by repeated injections of second vaccine in doses doubled at intervals of a fortnight, until mortality ceases. In this way repeated reaction may be elicited and a stronger immunity conferred.† In practice the effects of injection of quantities higher than 1 c.c. have not come under observation so far, since mortality has usually ceased after the second inoculation of second vaccine. If, however, it is definitely established by future observation that such inoculation with

* Cf. Dixon. Report, Acting Director of Agriculture, Cape of Good Hope, year 1905.

† Cf. Lukacs *loc. cit.* (20), and Goodwin, *American Veterinary Review*, Vol. XLIII, No. 3, 1913.

the strains at present used in preparing the vaccine is not sufficient to afford protection against certain unusually virulent local strains, the problem of polyvalent or multiple vaccines will have to be faced. A vaccine incorporating various local strains may have to be aimed at or special vaccines may have to be attempted for the districts in which strains of peculiar virulence are dominant. The task so set abounds with difficulties and demands much patient work, but the execution of Laboratory trials has already been commenced, and strains are being collected from various parts of the country from animals which break down in immunity after inoculation with the vaccine now in use.

But this is the future only from the vaccine-manufacturer's point of view. The various owners of stock throughout the country on whose farms Anthrax occurs also have their task. To them we would commend for consideration the following passage from McFadyean. "*M. Pasteur himself, before he had discovered the method of vaccinating with attenuated cultures, declared in very precise language his belief in the easy extinction of Anthrax ('Je crois fermement à la facile extinction de ce fléau')* by the exercise of proper care in disposing of animals dead of Anthrax. In support of this belief he cited a most instructive case, in which the annual loss from Anthrax on one farm had in ten years been reduced from a hundred animals to three, by the simple precaution of burying every Anthrax carcass in a special piece of ground fenced off so as to prevent the access of animals to it.*"

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APPENDIX.

Extract from the Stock Diseases Regulations, Union of South Africa. Special Regulations: Anthrax.

(a) An owner or person in charge of an animal suffering from Anthrax shall not permit any other person to have access to such animal unless he be a person whose access to the animal is necessary for the proper care thereof or a person entitled under these regulations or by Minister's orders to examine the animal.

(b) When an animal has died, or is suspected of having died of Anthrax, the owner or person in charge of the carcass shall cause the same to be properly burned, or, if burning be impossible, shall cause the carcass to be buried intact, and the burial place to be enclosed in such a way as to prevent stock from grazing over it.

(c) Any person who has been in contact with the excreta, discharges, or any portion of an animal which has died, or is suspected of having died of Anthrax, shall use the best available means of disinfecting his person and apparel.

(d) The person in charge of an animal suffering from Anthrax shall cause all excreta, litter, and discharges whatsoever therefrom to be buried or burned, and the places where such excretions or discharges have lain to be properly disinfected.

(e) It shall be the duty of the owner or person in charge of the animal which has been in contact with an animal affected with Anthrax to cause or permit such animal to be inoculated at the discretion of the Principal Veterinary Officer if that officer so require.

Investigations into Lamziekte in Cattle.

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IN the Third and Fourth Reports of the Director of Veterinary Research, Theiler, Green, and Viljoen* dealt more especially with the dietetic deficiency theories, and in particular with the vitamine hypothesis, as applied to Lamziekte. In this paper will be recorded the results of experiments and observations made during the last few years not only on the Experimental Station, Armoedsvlakte, but also on other farms in the lamziekte areas which were inspected by the writer.

As a result of our further extensive experience it is thought necessary to discuss several general aspects of the disease which had already been dealt with by previous writers, such as its symptomatology, animals affected, latent or "incubation" period, conditions under which Lamziekte is contracted, etc.

SOIL AND VELD CONDITIONS.

Under this heading will be considered only the geological conditions and types of veld on which the disease is contracted, attention being paid elsewhere to the different changes in the veld produced by unfavourable climatic conditions. On the Bechuanaland area Lamziekte is most prevalent on limestone and dolomite formations, but it also occurs on farms which are practically free from rock of any kind. Sandy soil, especially when shallow, or when mixed with stone, particularly limestone, seems to be the most common formation on which the disease occurs, and, in fact, it can be stated that on all farms in this area on which it is prevalent, the soil is of a sandy nature. Lamziekte is unknown or very rare on farms where the soil formation is a deep alluvial. As will be discussed later, the climatic conditions play an important part in the development of the disease, and it may be said that Lamziekte appears most commonly on farms where the soil formation is such that very little moisture is retained, so that plants growing on such soil quickly respond to changes in the climatic conditions. Thus, plants growing in shallow sandy soil, or over stony ridges, can be seen to sprout or grow very rapidly after a shower of rain and just as quickly wither and dry up after the rain has ceased and hot weather has set in again. It is chiefly for this reason that the disease occurs more rarely on alluvial soil, in vleis, near rivers, in bushveld, or, in fact, wherever a good deal of moisture is retained in the soil after rains. This factor was very clearly brought out in the Griqualand West area, where a particular farm remained practically free from Lamziekte

* Theiler, A., Green, H. H., and Viljoen, P. R.: Contribution to the Study of Deficiency Disease, with special reference to the Lamziekte problem in South Africa, Third and Fourth Reports of the Director of Veterinary Research, Department of Agriculture, Union of South Africa, 1915.

although all the neighbouring farmers were suffering heavy losses from the disease. Through the farm in question was running a large vlei, several miles long, and about a mile wide, on which the soil and vegetation differed markedly from that on the adjoining farms. Whereas the vegetation was dry and looked parched on the latter, the veld in the vlei was quite green and healthy looking. Signs of moisture were present everywhere, as evidenced by the abundance of vlei vegetation, i.e. vegetation which grows specially in moist places. The soil in the vlei was deep and contained a good deal of clay as compared with the sandy soil on the adjoining farms.

As regards the nature of the vegetation, it must be stated at the outset that *Lamziekte* occurs only on grass veld and has never been seen on Karroo veld. Naturally in the grass veld many species of shrub and plants other than grasses are encountered, but grasses predominate. Concerning the grasses, many farmers accuse "sour" veld as being the cause of the disease, but there is no clear definition of "sour" veld, so that what is known as a sweet grass in one district is called a "sour" grass in another, and vice versa. If "sour" grass is defined as a specially innutritious or unpalatable grass, it can be excluded altogether as a possible cause of the disease on the score that the majority of grasses on most farms in the Bechuanaland area have a very high nutritive value. It is probably not so much the species or type of grass that is involved in the production of the disease, as the unfavourable climatic and soil conditions to which the plants are exposed. The observation has been made repeatedly that the disease occurs very rarely, or not at all, on veld where the plant "Brak ganna" (*Salsola* sp.) grows abundantly, and, in fact, on farms where plots of this plant have become established it is the custom to allow cattle access to these as soon as the disease makes its appearance on the farm; it is said that in this way the disease is checked. With our present knowledge it is difficult to explain the rare occurrence of the disease on ganna veld on a farm which is situated in the centre of the lamziekte area. Some farmers state that the ingestion of this plant by animals produces a marked laxative, or even purgative, effect and that in this way poisons or toxins are not absorbed from the intestines. It is possible that here again the plant may grow only on deep, alluvial soil which retains moisture and on which *Lamziekte*, no matter what the nature of the vegetation is, is never very prevalent. The question is of sufficient interest to require further investigation and preparations are now being made to establish plots of ganna on the farm Armoedsvlakte.

CONDITIONS UNDER WHICH LAMZIEKTE OCCURS.

Elsewhere in this report it will be seen that although several theories regarding the cause of *Lamziekte* have been investigated, we have again had to fall back on the "grass toxin" theory so ably propounded by Theiler.* During the last few years the writer has made a special point of studying the conditions under which *Lamziekte* makes its appearance on a farm. These include climatic and atmospheric conditions (rainfall and temperature) on the one hand, soil and veld conditions on the other hand as influenced by the former.

* Theiler, A.: "Facts and Theories about Stijfziekte and *Lamziekte*," Second Report of the Director of Veterinary Research, Department of Agriculture, Union of South Africa, 1912.

These different factors appear to be so closely connected one with the other in the production of the disease that they cannot very well be discussed separately, and, in fact, they will all be referred to in different places of this chapter. Apart from observations on the veld in general, both on the experimental farm Armoedsvlakte, on other farms in the Bechuanaland area, and in other parts of the country, a closer study of these conditions was made in small paddocks on Armoedsvlakte in which several grazing experiments were being conducted. All these observations point to the cause of the disease originating from changes produced in the vegetation of lamziekte farms by the action of certain climatic influences, such as heat and drought. Lamziekte has always been recognized as a *disease of drought*, but a mere study of the rainfall over a long period is very deceptive, because it is not so much the total amount of rain as its distribution over a certain period that matters, although it must be clearly understood that the total annual rainfall on lamziekte areas is a comparatively very small one. Lamziekte occurs most commonly during the summer months, i.e. during the growth of vegetation, and the rainfall over lamziekte areas is very irregular, the rule being that a shower or showers of rain are followed by a spell of drought; if this happens in spring when the young plants show active growth, an interruption in growth takes place with the result that the vegetation shows a stunted appearance. During periods of drought when this interruption in the growth of vegetation is apparent, the disease becomes prevalent, and it is believed that under these conditions a toxin is produced in the grass. By studying the veld at this stage, one's attention is drawn immediately to the plants growing on sandy or shallow soil or on stony ridges where the least moisture is retained in the soil, and where consequently the stunted growth becomes apparent first. In this connection the excessive heat—the maximum temperature in the shade often rises to over 100° F.—undoubtedly plays an important part. The hot sun acting on plants growing on sandy or stony soil soon makes its effects felt, as evidenced by the drooping leaves of these plants after only one day's heat following a shower of rain. It can, therefore, be understood quite easily that the young grasses growing on veld that had been burned are influenced to a much greater extent by the sun, and hence it is quite a common occurrence for the disease to break out amongst cattle grazing over young "burns." In these "burns" the old plants and the natural humus covering the surface of the soil have been destroyed, allowing the sun's rays to act directly, not only on the young plants, but also on the soil itself, the result being the rapid evaporation of the moisture contained in the soil and early wilting and drying up of the young plants. The writer has had occasion to investigate several outbreaks of the disease on farms where the animals were dying on the young "burns," and where the outbreaks were checked by moving the animals on to "old" veld. As regards the observation that Lamziekte never or rarely occurs in winter, it is perhaps necessary to explain the reasons for this, in so far as the veld conditions are concerned. Sometimes a few cases of Lamziekte do occur in winter, and in this connection it must be explained that even in winter slight showers of rain very often fall, so that more than likely even in this season a certain amount of growth takes place in the vegetation. That this is so can be shown by the results of a grazing down experiment which was carried out in a small paddock (12 acres) in which a small

number of cattle (15) had been allowed to graze day and night for several months, the disease making its appearance in mid-winter on veld which had been grazed down completely, and which had been stimulated to growth by the constant grazing down process. Lamziekte is said to occur sometimes under the best veld conditions, the veld being described as luxuriant. Such an occurrence could be easily explained by the fact that the disease requires a certain period to develop; the length of this latent period has not been definitely established, but, as is shown elsewhere in this paper, it can be as long as twenty-one days, and possibly it may be much longer in some cases. When the disease makes its appearance, therefore, under the best of veld conditions, one must always refer back to the veld conditions which prevailed several weeks, at any rate, before the outbreak.

ANIMALS AFFECTED BY THE DISEASE.

Lamziekte attacks all bovines, irrespective of breed or sex, but young animals and cows in calf or in milk appear to be more susceptible. It is often said that trek oxen are not very susceptible to the disease, but this has not been my experience. On the ordinary farm, as a rule, the percentage of oxen to breeding cattle is a very small one, and hence losses amongst the latter are much more noticeable. For cases where the percentage of losses amongst oxen is really much smaller than that amongst breeding stock, one could easily find an explanation. It is a well known fact that change of veld, which frequently happens in the case of trek oxen, is one of the methods of combating the disease. Provided oxen are given the same treatment, as far as veld grazing is concerned, as breeding cattle, the percentage of losses amongst oxen will be found to be much the same as in breeding animals. Owing to the variation in the number of cattle running on the farm during any definite period, it is difficult to give absolutely accurate figures showing the incidence of the disease amongst cows, heifers, and oxen, but the following approximate percentages have been worked out for cattle on Armoedsvlakte during the period July, 1915, to January, 1918. The percentages were calculated by comparing the number of fatal cases amongst cows, heifers, and oxen to the actual number of the same class of animals running on the farm at different times during this period. The averages were then taken for the whole period, and the following are the percentages:—Heifers, 12 per cent.; cows, 11 per cent.; oxen, 9 per cent. As only very few tollies, i.e. young oxen not receiving exercise, came under consideration, they were included amongst the oxen. It will be seen, therefore, that heifers are slightly more susceptible than cows, but the difference is not great. Oxen are less susceptible than both cows and heifers, but this difference is not as marked as one would expect when considering the general opinion prevailing amongst farmers. Cattle in good condition are certainly more susceptible than animals not in good condition, and cattle in poor condition very rarely contract the disease. Some animals go down with poverty, and are then said by some farmers to be suffering from Lamziekte, but the symptoms shown by animals down with poverty are quite different to those seen in Lamziekte cases. The former feed, ruminate, and drink normally, and, in fact, appear to be in perfect health, excepting for their inability to keep on their legs. Lamziekte has been reported by farmers as occurring in other species of domestic animals, such as goats, sheep,

donkeys, and horses, but, with the exception of one suspicious case in a boer goat, the writer has never met with the disease in any species other than cattle. The goat in question suffered from a disease closely resembling Lamziekte in cattle, showing similar symptoms, running the same course, revealing similar post-mortem changes, and showing identical microscopic lesions in the muscles. It is difficult to express a definite opinion on the one case, but taking into consideration the experience of several farmers, particularly those in the Griqualand West area, the writer is inclined to the opinion that the disease does attack boer goats. It is not so certain that other breeds of goats are affected, as the disease was never seen amongst Angora goats which were running on Armoedsvlakte for many months. The same remark applies to Merino and Afrikander sheep, donkeys, and horses. In fact, it is extremely doubtful whether the disease attacks equines at all, as horse, mule, and donkey breeding, as far as Lamziekte is concerned, is carried out very successfully on Bechuanaland farms, where cattle farming is impossible on account of the severity of Lamziekte.

INCUBATION OR LATENT PERIOD.

It is a well known fact that when cattle are brought from a non-lamziekte farm to a lamziekte farm the disease does not attack these animals for some months after their arrival on the lamziekte farm, and this fact suggests a rather long period for the disease to develop in animals. It also lends strong support to the "cumulative toxin" theory so ably put forward by Theiler (*l.c.* p. 258). At different times during the last three years animals have been sent for experiments to Armoedsvlakte from farms in the Pretoria district where Lamziekte is unknown. As a rule these animals were used, at any rate for some time after their arrival, in plant feeding and grazing experiments, in which they did not have access to the general veld, but in the case of the grazing down experiments in paddock C, at any rate, some of these animals had access to veld where the disease was actually more prevalent than on other parts of the farm. Of the total of 72 animals which have arrived on Armoedsvlakte from Pretoria, 11, or a percentage of 15, have so far contracted the disease. In Table No. 1, details are shown regarding the mortality amongst these animals, and the periods they had been on a lamziekte farm before symptoms of the disease developed:—

Table No. 1.

Nos. of Animals concerned.	Date of Arrival on Lamziekte Farm.	Date on which Symptoms were First Seen.	Total Period on Lamziekte Farm before Symptoms Developed.
3251	13.12.14	17.10.17	2 years 10 months 4 days.
3387	5. 9.15	21. 8.16	11 months 16 days.
3384	5. 9.15	16. 2.16	5 " 11 "
3591	1.11.15	15. 7.16	8½ " 0 "
3593	1.11.15	10. 7.16	8 " 10 "
3602	7.11.15	3. 8.16	8 " 26 "
3606	7.11.15	20. 6.16	7 " 13 "
3610	7.11.15	29. 2.16	3 " 22 "
3614	7.11.15	16. 6.16	7 " 9 "
3878	25.11.16	15.10.17	10 " 20 "
3879	25.11.16	19.10.17	10 " 24 "

As will be seen, the shortest period in which symptoms became evident was 3 months 22 days, all other cases appearing after a much longer period.

For some time it has been suspected that Lamziekte has an "incubation" period, or, more correctly speaking, a latent period during which the toxin develops in the animal body and before clinical symptoms are exhibited by the animal. This "incubation" period was first suggested by the results of muzzling experiments carried out by Mitchell,* when two animals contracted Lamziekte 12 and 13 days, respectively, after they were muzzled. Recently attempts have been made to determine the period required for symptoms to develop, and for this purpose some kraaling and grazing experiments have been carried out. In these experiments 40 head of cattle were kraaled and fed on food-stuffs from lamziekte-free areas for a certain period, and after that they were allowed to graze over lamziekte veld for another period. By varying the periods of kraaling and grazing it was hoped to arrive at some definite data regarding the latent period of the disease. Unfortunately, however, the results obtained so far are not very definite, but the following information was gleaned:—Out of a batch of 40 animals two showed symptoms of Lamziekte on the third and one on the fourth day after the animals were placed in the kraal; three showed symptoms at different periods after returning to the veld, one on the eleventh day, one on the eighteenth, and one on the twenty-ninth day. The animals were kraaled for seven days and allowed to graze for thirty days, so that in the case of the latter three animals there is no certainty that the disease was contracted after they were returned to the veld for grazing. It is rather significant, however, that all three animals which died in the kraal showed symptoms of the disease within four days after they were brought in from the veld. This makes the "incubation" period unusually short, and one would be inclined to think that perhaps the complete change in diet shortened the usual developmental period in these cases. Much more light is thrown on this subject by a more recent case of Lamziekte which occurred in an animal twenty-one days after it was removed from veld grazing, stabled constantly, and fed on food-stuffs from a non-lamziekte area. Three days before the appearance of clinical symptoms this animal received by transfusion from an animal suffering from Lamziekte three litres of blood, and at that time one could not help suspecting that the disease was transmitted by blood transfusion. Since then the transfusion experiment has been repeated on a very large scale, with negative results in all cases. The only possible conclusion one could arrive at, therefore, was that the animal contracted the disease naturally before it was stabled, and that clinical symptoms only appeared twenty-one days afterwards. It is also possible that the disturbance in metabolism, which must have been caused by such a large quantity of blood, brought out, or assisted to bring out, a latent form of the disease. As a result of these observations and those of Mitchell (*l.c.* p. 362) one could conclude safely that Lamziekte has an "incubation" or latent period, which varies greatly, but which can be as long as twenty-one

* Mitchell, D. T.: "Lamziekte," Second Report of the Director of Veterinary Research, Department of Agriculture, Union of South Africa, 1912.

days; in the case of Pretoria animals, this period appears to be much longer.

SYMPTOMATOLOGY.

The symptoms exhibited by animals suffering from Lamziekte have been described in great detail by previous workers on this disease, and hence in this paper it will not be necessary to elaborate on the description of symptoms, excepting in so far as the different types of the disease are concerned. There is no definite dividing line between the different forms of the disease, excepting that the symptoms develop more rapidly, and that death takes place sooner in the one type than in the other. During these investigations the cases of Lamziekte have been divided clinically into the following types:—(1) Peracute, (2) Acute, (3) Subacute.

Premonitory Symptoms.—As a rule, in all forms of the disease the symptoms appear suddenly, and no premonitory signs are observed. That does not mean, however, that no such signs are ever present. In our methods of cattle farming in this country the animals are allowed to run on the veld, and are not kept under close observation, so that even if premonitory symptoms were present, they would probably not be noticed. It is only when animals are kept under close observation, and are inspected several times a day, that one could expect to see the very early stages of Lamziekte. A few such cases came under my observation in one of our small paddocks, where the animals were inspected at least twice a day. The premonitory symptoms in these cases were not very characteristic, and one certainly could not say definitely that the animals were going to develop Lamziekte. Regarding these cases, the following notes were made at the time:—

“A further very interesting occurrence was that in all three animals we suspected that there was something wrong two days before definite symptoms of illness appeared. In every case the animal did not seem to be in normal health, but it was most difficult to give a description of any departure from the normal. It was only because the animals were kept under such close observation that the slight departure from normal could be noticed. It is highly probable that premonitory symptoms would also be detected in other cases of Lamziekte if the animals could be kept under the same constant observation.”

These symptoms consist of very slight uneasiness, which would not be detected unless the normal habits of the affected animal were well known; only persons with much experience of the normal habits of cattle would, therefore, be able to detect the slight departure from the normal. The writer cannot agree with other observers that signs of Pica, craving for bones, etc., can be included in the premonitory symptoms of Lamziekte. This craving for bones, etc., by cattle has been observed by me during an extensive experience with cattle farming in this country on farms where Lamziekte has never been known to exist. It is possibly an indication of sour veld, and can be stopped to some extent by giving the animals a salt lick. On Armoedsvlakte an excessive craving for bones has not been noticed, and, in fact, in one small paddock where carcasses were strewn broadcast we had great difficulty in getting the animals to chew the bones.

(1) *Peracute Lamziekte*.—As calculated on 50 animals that died from Lamziekte on Armoedsvlakte, the percentage of peracute cases works out at 18. The duration of this form of the disease is very short, probably only a few hours, and, in fact, all cases of the disease which were found dead within twenty-four hours after the animals were last seen alive and in good health, or which were visibly ill for only a few hours, have been put down to the peracute form. The general rule is that animals attacked by the peracute form of the disease are found dead in the veld and had never been noticed to be ill, thus resembling Anthrax in its sudden development. Only one case of peracute Lamziekte has come to my notice where the opportunity of studying the symptoms presented itself. This animal was noticed ill six hours after it left the kraal in apparently good health, the symptoms being those of a very acute attack of Lamziekte, viz., very marked muscular weakness and inability to rise almost immediately after the onset of symptoms, and death took place within six hours from the time the first symptoms were seen.

(2) *Acute Lamziekte*.—This is the most common form in which the disease has been observed by the writer in the Bechuanaland area, about 80 per cent. of all cases being of this type. Its onset is also very sudden, but muscular weakness is only slight in the very early stages. At the beginning the animal will be noticed to be rather dull, not feeding, and inclined to isolate itself from the main herd. The patient appears to be uncomfortable, sometimes excited, and soon muscular weakness can be noticed, when the inclination to lie down frequently will be observed. On being driven, stiffness will be noticed in the forequarters, the patient showing a sluggish gait, and the tendency to lie down becomes more marked, indicating increasing muscular weakness. This stage is very characteristic, and the animals can be described as showing the typical Lamziekte gait. Later on, progression becomes more difficult, weakness is shown also in the hindquarters, and from a few hours to a few days the animal goes down and is unable to rise again; when assisted on to its legs the animal is unable to maintain itself in the standing position. The patient's muzzle becomes very dry and caked with dry crusts, and faeces of varying consistency covered with mucous material are passed in small quantities. The temperature in all cases of Lamziekte remains normal throughout the course of the disease. The beast may lie in the sternal position for a day or two, but after that progressive weakness makes it impossible for the animal to maintain even the sternal position, and the patient goes down stretched out on its side, death supervening soon after. Paralysis of the tongue and throat muscles has been observed in several cases which, as a rule, proved to be rapidly fatal. In these animals salivation is profuse, the jaws are moved constantly as if the animal was ruminating, the tongue appears to be limp, and swallowing has become impossible. Some writers and many farmers make the statement that animals suffering from acute Lamziekte will be found to eat and drink normally, but this has not been the experience of the writer. Eating, and especially ruminating, is almost entirely suspended, and water is partaken of rarely, and then only in small quantities. Sometimes the patient will be seen to take up and nibble at food, but this food will get no further than the mouth. The act is really only a pretence at eating, and the movement of the jaws is mistaken for rumination. Sensibility in the

whole body remains unimpaired right up to near the point of death, and voluntary movement of the limbs is also maintained, excepting that there is marked muscular weakness. Acute cases of Lamziekte last from twelve hours to five or six days, average two to three days, and the vast majority of cases prove fatal.

(3) *Sub-acute Lamziekte*.—This type of the disease is not very common, and only claims 6 per cent. of cases, as worked out on 50 cases which have occurred on Armoedsvlakte recently. It differs markedly from sub-acute or chronic cases described by previous observers. The writer, who has probably seen close on 200 cases of Lamziekte, has never met with cases of the disease described by other authors, where the animal lies down for several months, is unable to get up, feeds and drinks normally, and in fact appears to be in normal health, excepting for its inability to rise to its feet. Although there is not the slightest desire to cast any doubt on previous observations, it has come to my notice frequently that farmers call ordinary cases of poverty chronic Lamziekte. In these cases it may be possible that the animal had suffered from an initial attack of Lamziekte, which left it in a rather exhausted condition, but this is extremely doubtful. Many farmers state that the chronic or paralytic type of Lamziekte used to be the most common form in which the disease manifested itself. The sub-acute form, as seen by the writer, differs from the acute in that the onset is not so sudden, and that the course of the disease is much more prolonged. At first only slight stiffness in the forequarters is noticed, but, compared to acute cases, this stiffness or muscular weakness increases only very slowly. The animal does not appear very ill, drinks normally, and eats fairly well, although in some cases the appetite was very small, and in others almost entirely absent. As in acute cases, the animal lies down more frequently than one in normal health, but there is no difficulty in rising except in the later stages. After the animal has gone down, which happens from the fourth to the fourteenth day, death takes place in from two to seven days. Nearly all cases which go down and are unable to rise terminate fatally. Some animals never go down altogether, and these cases frequently recover.

Post-mortem Findings.—The changes found in carcasses of animals which have died from Lamziekte have been described in detail by many earlier investigators, but in order to make this report more complete a short summary of the more important post-mortem lesions will be given. These lesions do not vary very much in the different types of the disease, excepting that in the subacute forms mucosenteritis is more marked. At the outset it is necessary to state that, as a rule, no very marked pathological lesions can be seen macroscopically, but the following changes are fairly constantly present:—Hyperaemia of the abdominal organs, of the mucosa of the fourth stomach and intestines, quite commonly ecchymoses in the epicardium and endocardium, and in many cases hyperaemia of the lungs. *The subcutaneous tissues* are quite normal, excepting in cases where the animal received bruises as the result of falling down or struggling to get up, and also oedematous infiltration in cases where the animals have been lying down for some time. *The liver* shows a rich blood supply, and evidence of fatty degeneration in some cases. *The gall*

bladder in most cases is distended with bile of a dark green colour, and rarely turbid. *The spleen* also shows a rich blood supply, and in most cases hyperaemia is present; no marked pathological changes are ever present. *The kidneys* never show any changes other than hyperaemia, especially of the boundary zones. *The abomasum and intestines*, particularly the small bowels, always show the presence of some hyperaemia, which, in some cases, may be only slight and patchy, but in others diffuse and very marked. In addition muco-enteritis, particularly of the large intestines, is present in many cases, and sometimes is very marked, the mucosa being much swollen and wrinkled, and containing abundant mucous discharge. As a result of this condition, the sick animal is often noticed to pass faeces covered with a film of mucous material. In the abomasum old scars covered with coagulated blood are seen in some cases, and in a few animals the mucous folds have been found missing; no signs of inflammation were present, and hence the absence of the folds was ascribed to a congenital origin. *Third stomach or omasum*.—In 100 autopsies this bowel was found to be altered in only 25, the changes occurring in its food contents, which were hard and dry in some cases (impaction), and only slightly drier than normal in others. In view of the fact that some farmers call Lamziekte nothing else but a form of impaction of the omasum, this statement is important. The writer considers the impaction occurring in some cases only a sequel to digestive derangement, which is undoubtedly present in cases of Lamziekte. *Rumen and reticulum* are normal in all cases, but, as is commonly the case in most parts of this country, foreign bodies, such as pieces of bone, nails, etc., are sometimes found in the contents of the reticulum. *Mesenteric glands* are normal in all cases, but in few animals, just as in other diseases, they are swollen and slightly oedematous. *Genital organs* are always normal. *The urinary bladder* is normal, excepting in cases which had been down for some considerable time, when sometimes the bladder is much distended with urine.

Thoracic cavity.—The pleurae remain mostly unaltered, but in a few cases blood extravasations have been seen on the mediastinal pleura. *Pericardial cavity* very often contains an increased quantity of fluid, which may be quite clear, but sometimes is blood-tinged, or may contain coagula. *Epicardium*.—In the majority of cases petechiae can be seen, but these vary in size and in their distribution, and are most commonly observed along the course of the coronary vessels. *The heart* as a whole is altered in only the minority of cases, when the course of the disease has been rather prolonged, the heart in these cases being rather flabby and dilated.

Endocardium.—In most cases blood extravasations are present, particularly in the left ventricle, and nearly always involve the area to which the valves are attached.

Myocardium appears normal in most cases, but in a few animals it was found to be rather pale in colour, soft in consistence, and showing signs of fatty degeneration.

The lungs in most autopsies show hyperaemia, which is accompanied by oedema, and sometimes blood stasis, but these changes are undoubtedly produced just prior to or during the death of the animal.

as they are absent in post-mortems made on animals that were killed while suffering from the disease. *The pharynx* very often shows signs of hyperaemia. *The larynx and trachea* are normal, excepting for the presence of petechiae on the mucosa of rare cases. *The thyroid glands* show hyperaemia in their substance in only rare cases. *The pharyngeal* and other lymphatic glands are normal in most cases, but rarely are swollen and oedematous, just as in the mesenteric glands. *The brain* itself has never been found to exhibit any marked abnormalities. The blood-vessels of the meninges in nearly all cases are injected or congested, but this is considered to be in the nature of blood stasis occurring at the time of death. In some cases the pia-mater shows a greyish or bluish-grey discoloration, but no importance can be attached to this pigmentation which is also present in animals which died from causes other than Lamziekte. *The spinal cord*.—No definite alterations have been established in the spinal cord and its membranes. In some cases there appears to be an increase in the quantity of subarachnoidal fluid, but this fluid is difficult to measure with accuracy, and the amount of fluid in different animals varies to an enormous extent. It can be stated definitely, however, that a marked increase of subdural fluid is not constantly met with in lamziekte animals. *The large blood-vessels* are normal, excepting that in some old animals lesions of arterio-sclerosis are seen in the intima of the aorta, particularly in its abdominal portion.

The nasal cavities.—The mucous membrane covering the septum nasi and turbinated bones has very often been found to be rather congested, but this membrane is normally very vascular, so that possibly the congestion only takes place at the time of death of the animal. *Musculature*.—No naked-eye lesions have ever been detected in the muscles by the writer, and the inter-muscular connective tissues and fats always appear normal. *The bones*.—No naked-eye lesions or abnormalities have been detected in any bones of the lamziekte skeleton.

GRAZING DOWN EXPERIMENTS.

During the last few years attempts have been made to locate Lamziekte in small camps or paddocks, the object being to facilitate the study of plant life, etc., on the smallest area possible. At first we were able to locate the disease in a 100-acre camp, which we afterwards succeeded in dividing into four smaller camps, each 25 acres in extent. By continuing the grazing experiments we were able eventually to get cases of the disease in paddocks only about 8 acres in extent. A further object of this series of experiments was to find out the conditions under which Lamziekte was contracted, and naturally by making observations on small areas we were hopeful of finding a clue leading to the discovery of the cause of the disease. The results of feeding experiments carried out by Theiler, Green, and Viljoen (*l.c.*, p. 257) indicated that supplementary rations did not in any way reduce the incidence of Lamziekte amongst cattle grazing over lamziekte veld, and hence in this series of experiments it was possible to keep the small areas grazed down completely over comparatively long periods by giving the experimental animals maintenance rations. In

all these experiments the animals were kept constantly in the small camps where they received their drinking water in troughs.

Experiment No. 1.—On 25th July, 1915, twelve head of cattle were placed in paddock "B," 25 acres in extent, when the grazing was plentiful.

Cases of Lamziekte.—(1) Ox No. 2455 died of peracute Lamziekte on 14th August, 1915, i.e. only twenty days after it was put in the experiment. (2) Ox No. 2266 died of peracute Lamziekte on 17th September, 1915, or less than two months after the experiment was started. (3) Cow No. 2602 died on 21st September, 1915, from peracute Lamziekte, or within two months from the time the experiment was commenced. This case was interesting in that the animal had passed through two previous attacks of the disease. (4) Tollie No. 2434 showed symptoms of Lamziekte on 23rd September, 1915, and died two days later. This animal was placed in the experiment on 3rd September, 1915, to replace another, so that symptoms of the disease appeared twenty days after the animal was placed in the experiment. From 9th October, 1915, the animals received a supplementary ration as the grazing was getting scarce, and on the 3rd January, 1916, the experiment was discontinued.

Remarks.—Four out of twelve animals died from Lamziekte, while on the remainder of the farm the mortality was a very small one. Two animals showed symptoms of the disease twenty days after they were placed in the experiment, and, as the disease was not prevalent on other parts of the farm during the same period, it appeared as if the disease was contracted in this paddock. The animals were noticed to be very fond of grazing over a certain ridge on which the predominant grass was *Chrysopogon serrulatus*. This grass was found to be badly infected with a parasitic fungus (*phyllachora*), which forms the subject of another experiment.

Experiment No. 2.—Carried out in sub-division 1, paddock "C," 12 acres in extent, with eight animals sent from a non-lamziekte area. The experiment was commenced on 2nd November, 1915, and concluded on 6th May, 1916. No cases of Lamziekte occurred.

Remarks.—From 4th January, 1916, it was considered necessary to give these animals a supplementary ration, which consisted of 2 lb. crushed mealies, 1 lb. bran, Pretoria hay *ad lib.*, each per day. These animals came from a non-lamziekte area and, as such animals do not appear to contract the disease until they have been exposed to lamziekte veld for some months, it is possible that the experiment was discontinued too soon.

Experiment No. 3.—On 3rd January, 1916, eight head of cattle were placed in sub-division 2, paddock "C," about 13 acres in extent. In one corner of this camp a large number of carcasses from animals which had died from Lamziekte had been deposited, so that the animals had access to these carcasses.

Cases of Lamziekte.—(1) Heifer No. 3610 showed symptoms of Lamziekte on 1st March, 1916, from which it recovered eventually.

Symptoms of the disease were observed in this animal three months three weeks after its arrival from Pretoria and two months after it was placed in the experiment. (2) Calf No. M. 3614 developed symptoms of Lamziekte on 1st March, 1916, and died the same night. This case was interesting in that the animal was a young calf, only four months old, still unweaned. The calf was developed very well for its age, however, and was grazing almost like a full-grown animal. This experiment was discontinued on 6th May, 1916.

Remarks.—From 16th February, 1916, very little grazing was left in the paddock, and hence the animals received supplementary rations. When symptoms of the disease occurred the animals had access to only short tufts of grass, and consequently had to live almost entirely on the supplementary rations. This result is most interesting from the point of view of the deficiency theories, and will be mentioned again later in this report. The animals also had access to lamziekte carcasses, but they were never noticed to interfere very much with them. Although no definite suspicion could be thrown on the presence of these carcasses as a possible source of "infection," they were made the subject of another experiment.

Experiment No. 4.—On 6th May, 1916, the dividing fence between sub-divisions 1 and 2 of paddock "C" was removed, and the two batches of animals running in these sub-divisions were allowed to run together and form one experimental lot. The object was to allow the animals more space in which to roam about and so prevent too much tramping down of the veld. From the commencement of the experiment the animals had to receive supplementary rations, because the grazing area was already much grazed down.

Cases of Lamziekte.—(1) Cow No. 3614 showed symptoms of acute Lamziekte on 17th June, 1916, and died the following day. This animal arrived from Pretoria on 7th November, 1915, since when it had been in experiments Nos. 1 and 3. It had been on a lamziekte farm, therefore, for over seven months before it showed symptoms of the disease. (2) Heifer No. 3610 showed symptoms on 17th June, 1916, and died the following day. This animal was referred to in Experiment No. 3 as having recovered from an attack of the disease, and it is interesting to note, therefore, that it succumbed to a second attack within three and a half months from the first. (3) Heifer No. 3606 showed symptoms on 20th June, 1916, from which it recovered eventually. This animal also came from Pretoria on 7th November, 1915, and, like the preceding two, passed through experiments Nos. 1 and 3.

Remarks.—The mortality from Lamziekte was very high when compared to that amongst animals in other experiments. The disease made its appearance when the paddock was grazed down completely, only small tufts of grass remaining. By the constant close grazing and overstocking the growth of grass seemed to be stimulated, with the result that green sprouts could be seen here when no growth of vegetation could be detected on other parts of the farm. The young sprouts were continually being eaten down by the cattle, besides being dried

up by the sun during the day and frost at night. There was, therefore, a great deal of interference with the normal growth of these grasses, so that a stunted appearance was the result.

Experiment No. 5.—On 2nd July, 1916, this experiment was commenced in sub-division 2, about seven acres in extent, of paddock "C," which was sub-divided into four portions at this stage. At the commencement this area was very much grazed down, but the main object of the experiment was to find out whether cattle would still contract Lamziekte when exposed to veld which had been kept grazed down over a long period. In this sub-division no carcasses were present. The number of animals in the experiment was 14.

Cases of Lamziekte.—(1) Heifer No. 3593 showed symptoms on 10th July, 1916, i.e. about eight months after its arrival from Pretoria, and died two days later. (2) Heifer No. 3591 was first noticed ill on 15th July, 1916, and died two days later. This animal died from Lamziekte eight and a half months after its arrival from Pretoria.

Remarks.—Both cases of Lamziekte occurred within a fortnight after the new sub-divisions were made, so that there is a possibility of the disease having been contracted before this experiment was commenced. Both animals had access to lamziekte carcasses in a previous experiment, so that the results are somewhat complicated by this factor. In this case again the disease made its appearance amongst animals having practically no veld grazing, and which had to subsist almost entirely on supplementary rations.

Experiment No. 6.—Paddock "D" was divided into two unequal parts, and in the smaller portion, sub-division 2, about 10 acres in extent, this experiment was started on 25th August, 1916, with 15 head of cattle. The main object was to find out whether cattle would contract Lamziekte on veld which had been grazed down by a large number of sheep, and which was subsequently kept grazed down by cattle. About 160 sheep were allowed to graze over the area from 1st July, 1916, to 25th August, 1916, when they were replaced by the cattle. From the commencement the cattle had to receive supplementary rations, as the area was much grazed down by the sheep.

Cases of Lamziekte.—(1) Heifer No. 3120 was noticed ill on 22nd January, 1917, after having been in the experiment for five months, and died seven days later.

Remarks.—There is no certainty that the disease was contracted in this camp, as, unfortunately, owing to the shortage of Pretoria hay, all the animals had to be turned out into another camp for the period 5th September, 1916, to 11th September 1916, and 13th January, 1917, to 18th January, 1917. During the period of this experiment the disease was not very prevalent on other parts of the farm, and hence no definite conclusions could be drawn from the results. From the farming and botanical point of view, very interesting results were obtained. All the grasses were eaten close to the ground by the sheep, excepting the "steek" grass (*Aristida congesta*), which on account of its unpalatability, was left standing. The seeds from this grass were cast on the ground, and have since formed new growth

almost over the whole area, thus converting what was previously mixed grass veld into *Aristida* veld, or veld in which *Aristida* predominates.

Experiment No. 7.—Sub-division 4, paddock "C," is situated in one corner of this paddock, and in it a large number of carcasses from animals that had died from Lamziekte were placed. The area was only a small one, comprising about four acres, and hence from the commencement of the experiment, on 25th August, 1916, the animals received supplementary rations. The experiment was conducted with eight animals, which remained in the paddock constantly until 16th February, 1917. To ensure picking up of bones, ingesta, etc., from these carcasses by the experimental animals, a very small area was selected, and the remains of the dead animals were strewn over the surface of the whole area.

No cases of Lamziekte occurred.

Remarks.—As no deaths from Lamziekte occurred in this experiment, it is doubtful whether the disease would be contracted by animals consuming bones and other remains of animals which have died from Lamziekte.

Experiment No. 8.—The object of this experiment was to find out whether Lamziekte would be contracted in a small paddock in which the veld had been previously grazed down by a large number of cattle. Sub-division 1, paddock "B," about 12 acres in extent, was selected for this experiment on 29th July, 1916, when 50 head of cattle were used for grazing down the veld. On 25th August, 1916, these cattle were taken out and replaced by 15 others, which remained in the paddock constantly until the experiment was discontinued on 6th February, 1918. During the whole period of the experiment the 15 animals received daily supplementary rations, the natural grazing available to them being extremely limited. No cases of Lamziekte occurred.

Remarks.—During the same period Lamziekte was not prevalent on the farm, so that no definite conclusions could be drawn from the negative results.

Experiment No. 9.—The object of this experiment was to find out whether Lamziekte would still be contracted on a known "infected" area when such an area was overstocked with a large number of cattle, i.e. when the toxin was distributed amongst a large number of animals, thus lessening the chances of individual animals ingesting sufficient toxin to produce symptoms of the disease. On 31st August, 1916, 30 head of cattle were placed in sub-division 1, paddock "C," about 7 acres in extent, and kept there constantly. The experiment was continued until the veld had been grazed down completely and the animals could no longer subsist on the natural grazing. This condition was produced on 17th October, 1916, when the experiment was discontinued. No cases of Lamziekte occurred.

Remarks.—In this case again no definite conclusions could be drawn from the negative results, as the experiment could not be carried on long enough.

Experiment No. 10.—For some time past we have been suspecting a grass toxin to be the cause of the disease, but up to the present we have failed to produce cases of Lamziekte by feeding animals on grasses collected from the veld. In this experiment, therefore, it was decided to find out whether the disease could not be produced by allowing animals to graze over a small area of veld from which all plants other than grasses had been removed. Sub-division 1A, paddock "B," only about 3 acres in extent, was prepared for this experiment, and on 6th February, 1918, twelve head of cattle were placed in it. The area then was quite free from plants other than grasses, and since then it has been kept in the same condition by having natives constantly at work digging out any plants that may show above the surface of the ground. Up to the time of writing no cases of Lamziekte have occurred, but the disease has been practically absent from other parts of the farm during the same period. The experiment is being continued.

Experiment No. 11.—This experiment is being carried out on exactly similar lines as those just described for Experiment No. 10, excepting that it is being conducted on a much larger scale, the area cleared of plants other than grasses being about 10 acres in extent, and carrying 18 head of cattle. Whereas in the previous experiment the veld has been grazed down previously, in this experiment, which is carried out in sub-division 1, paddock "D," the veld affords quite natural grazing, no animals having had access to it for a period of a few years. The experiment was commenced on 6th February, 1918, and is being continued. No cases of Lamziekte have occurred so far.

DISCUSSION AND CONCLUSIONS.

As all these experiments were carried out with much the same object in view, it is perhaps advisable to discuss together and summarize the results obtained. Positive results were obtained in the following experiments:—Experiment No. 1, 4 cases; Experiment No. 3, 2 cases; Experiment No. 4, 3 cases; Experiment No. 5, 2 cases; Experiment No. 6, 1 case. Regarding the veld conditions, it can be pointed out that at the time when cases of Lamziekte occurred entirely different conditions prevailed in the first experiment as compared with those in the remaining experiments. In the former grazing was plentiful when cases of Lamziekte occurred, and after the veld had been eaten down considerably, no further animals contracted the disease. Whether further cases would have occurred if the experiment had been continued sufficiently long cannot be stated definitely. In all the other experiments losses from the disease occurred only when the veld had been grazed down considerably, and when only short tufts of grass remained. The veld conditions under which Lamziekte made its appearance in these experiments were most interesting. In 1916 the last rains fell during the first half of April, after which the weather remained dry until October. Most of the Lamziekte cases occurred in June and July, when the soil was very dry and growth of vegetation on the farm generally was at a standstill. In these small paddocks, however, entirely new veld conditions were created by the constant grazing down system; after the old grasses had

been eaten off, young green sprouts could be seen in these paddocks. These young sprouts were constantly being eaten by the cattle, and this seemed to stimulate their growth still further. Owing to the rather cold weather prevailing at the time, and to the dry condition of the soil, growth of vegetation was never very rapid, and, in fact, was repeatedly interrupted by cold spells, alternated by hot, sunny days. Under these peculiar climatic conditions, it is believed that grasses of a very toxic nature were produced, and that to the presence of a toxic principle in the grass the occurrence of the disease could be attributed. During the same period practically no growth in vegetation could be detected on other parts of the farm, and, in keeping with this, very few cases of Lamziekte made their appearance. Apart from these veld conditions, another factor, the presence of lamziekte carcasses in the small camps, must be taken into consideration. In Experiment No. 1 no carcasses were present on the grazing area, and yet four cases of Lamziekte occurred. In reviewing the experiments carried out in paddock "C," at one corner of which the carcasses were deposited, we find the following results:—In Experiment No. 2 the animals had no access to carcasses, and no cases of Lamziekte occurred. In Experiments Nos. 3 and 4 the animals had access to carcasses, and several cases of Lamziekte occurred. In Experiment No. 5 no carcasses were present, but the animals which died from Lamziekte had access to carcasses a fortnight before they were drafted into this experiment. In Experiment No. 7 the animals had access to a large number of carcasses, and yet no cases of Lamziekte occurred, although the experiment was continued for nearly eight months. It must be admitted that the first results made one suspect the lamziekte carcasses as having had some connection with the production of the disease, but this suspicion was completely upset by the results from Experiment No. 7. In the transmission experiments carried out by Mitchell (*loc. cit.* p. 262) and Walker,* attempts were made to transmit Lamziekte from sick and dead animals to healthy cattle, almost every conceivable part of the lamziekte carcass being used in these experiments, but the results were negative in all cases. In the experiments here reported on, the animals were never seen to take much notice of the carcasses, in spite of the fact that very often they were observed to lie down between, and even on top, of the carcasses. Moreover, it has not been the custom on Armoedsvlakte, for the last five years at any rate, to allow carcasses of dead animals to lie about on the veld, so that not many bones will be found in the larger camps, where Lamziekte is contracted just the same. A further very interesting result, especially so when considered in connection with the so-called deficiency theories, was obtained from these experiments. As previously mentioned, in some of the experiments the disease was at its worst when practically no natural grazing remained, and when, consequently, the animals had to subsist almost entirely on the supplementary rations which were supplied to them daily, and which consisted of food-stuffs obtained from farms where the disease was unknown. Under these conditions it does not seem possible that the disease could have been contracted as the result of a deficiency of any

* Walker, James: "Investigations into the Disease Lamziekte in Cattle," Second Report of the Director of Veterinary Research, Department of Agriculture, Union of South Africa, 1912.

kind in the lamziekte veld. From the results of these experiments one could, therefore, draw the following conclusions:—(1) Lamziekte is not a disease which can be associated with a deficiency of the ordinary food elements in lamziekte veld. (2) The disease can be contracted even when the bulk of the animals' diet consists of food-stuffs imported from an area where Lamziekte is unknown. (3) Lamziekte is contracted under certain peculiar veld conditions, where the vegetation shows an unhealthy, stunted growth due to unfavourable climatic and soil conditions.

*PLANT FEEDING EXPERIMENTS.

In this series of experiments methods will be described whereby attempts were made to produce Lamziekte in cattle by feeding them on different plants collected from the veld. In this way two theories were tested, viz.:—

- (1) The plant poison theory.
- (2) The grass toxin theory.

In these experiments the animals were tied up constantly, excepting for a few hours every day, when they were allowed to run out for exercise, during which they were muzzled carefully, wire muzzles being used of a mesh sufficiently small to prevent natural grazing of any kind.

Experiment No. 12.—On 26th November, 1916, nineteen head of cattle were drafted into this experiment, each receiving several plants per day, careful records being kept of the species and the quantities of plants fed to each animal. During the period 26th November, 1916, to 14th August, 1917, 140 plants other than grasses were fed to these animals. In this experiment an attempt was made to test practically every plant on the farm, as we were anxious to prove or disprove the existence of a poisonous plant which would produce the symptoms of Lamziekte. Needless to say, it proved to be an undertaking of some magnitude, but we succeeded in testing most of the plants growing on this farm. For the determination of these plants the writer is indebted to Mr. Pole-Evans, Chief of the Division of Botany. As a supplementary ration, the animals received a daily allowance each of 2 lb. mealies, 1 lb. bran, and grasses collected from the veld *ad lib.* Hence it will be seen that a grass feeding test was carried out at the same time as the plant feeding experiment. There was no real objection to this procedure, because, if a case of Lamziekte was produced, either the plants or the grasses could have been eliminated by further tests. The mealies and bran were used not so much for their feeding value as for mixing with plants to render them more palatable. In order to make a proper mixture, the plants were finely chopped up before being mixed with the mealies and bran. Details of the feeding tests are shown in Table No. 2, which requires no further explanation, excepting that a few plants occur, the names of which could not be given, as no suitable material for their determination was available to the Division of Botany.

* The feeding experiments formed part of the investigations into the cause of Lamziekte undertaken by co-operation with the Division of Botany (I. B. Pole Evans) with the ulterior object of testing some of the plants on their toxicity and others on their nutritive value.

TABLE NO. II.
Plant Feeding Experiments.

No.	Name or No. of Plant.	No. of Animal.	Total Period of Feeding.	Total Quantity Plant fed.	Result.
1	<i>Aerva leucura</i>	Heifer 3601 " 3327	94 days 96 "	36 lb. 5 oz. 8 lb. 0 oz.	Negative. "
2	<i>Alternanthera achyrantha</i>	Heifer 3604 " 3371	99 days 8 "	33 lb. 13 oz. 3 lb. 3 oz.	Negative. "
3	<i>Amaranthus Thunbergii</i>	Heifer 3402	78 days	31 lb. 15 oz.	Negative.
4	<i>Anthospermum rigidum</i>	Heifer 3875 " 3876	86 days 24 "	32 lb. 12 oz. 10 lb. 11 oz.	Negative. "
5	<i>Anthericum Macowani</i>	Heifer 3876	75 days	8 lb. 14 oz.	Negative.
6	<i>Anthericum</i> (spec. ?).....	Heifer 3877	81 days	15 lb. 9 oz.	Negative.
7	<i>Anchusa riparia</i>	Heifer 3385 " 3604	94 days 15 "	34 lb. 10 oz. 5 lb. 12 oz.	Negative. "
8	<i>Anthericum pilosum</i>	Heifer 3327	45 days	9 lb. 1 oz.	Negative.
9	<i>Aptosimum albomarginatus</i> ..	Heifer 3880	75 days	27 lb. 11 oz.	Negative.
10	<i>Aptosimum</i> (spec. ?).....	Heifer 3327 " 3397	86 days 24 "	30 lb. 1oz. 8 lb. 2 oz.	Negative. "
11	<i>Arthrosolen sericocephalus</i> ..	Heifer 3878	105 days	45 lb. 3 oz.	Negative.
12	<i>Artemisia afra</i>	Heifer 3878	81 days	10 lb. 4 oz.	Negative.
13	<i>Asclepiadaceae</i>	Heifer 3601 " 3385	75 days 78 "	20 lb. 5 oz. 20 lb. 2 oz.	Negative. "
14	<i>Bergia</i> (spec. ?).....	Heifer 3874 " 3375	97 days 15 "	35 lb. 10 oz. 7 lb. 9 oz.	Negative. "
15	<i>Blepharis molluginifolia</i>	Heifer 3876	104 days	12 lb. 8 oz.	Negative.
16	<i>Boerhaavia pentandra</i>	Heifer 3874	26 days	1 lb. 12 oz.	Negative.
17	<i>Borraginaceae</i> (spec. ?).....	Heifer 3877	75 days	25 lb. 1 oz.	Negative.
18	<i>Bouchea pinnatifida</i>	Heifer 3611 " 3383	86 days 24 "	28 lb. 9 oz. 8 lb. 0 oz.	Negative. "
19	<i>Brachystelma</i> (spec. ?).....	Heifer 3880	74 days	6 lb. 2 oz.	Negative.
20	<i>Bulbine asphodeloides</i>	Heifer 3604 " 3371 " 3082 " 2724	86 days 24 " 44 " 90 "	36 lb. 15 oz. 11 lb. 8 oz. 11 lb. 4 oz. 10 lb. 12 oz.	Negative. " " "
21	<i>Cassia mimosoides</i>	Heifer 3611 " 3082	36 days 60 "	7 lb. 11 oz. 12 lb. 4 oz.	Negative. "
22	<i>Cephalandra</i> (?).....	Heifer 3879	81 days	11 lb. 13 oz.	Negative.
23	<i>Cephalandra sessilifolia</i>	Heifer 3397	93 days	27 lb. 10 oz.	Negative.
24	<i>Chenopodium botrys</i>	Heifer 3874	80 days	26 lb. 0 oz.	Negative.

TABLE NO. II—(continued).
Plant Feeding Experiments—(continued).

No.	Name or No. of Plant.	No. of Animal.	Total Period of Feeding.	Total Quantity Plant fed.	Result.
25	<i>Chenopodium murale</i>	Heifer 3611	78 days	35 lb. 14 oz.	Negative.
26	<i>Chrysocoma ciliata</i>	Heifer 3371 " 3611	94 days 15 "	26 lb. 6 oz. 5 lb. 8 oz.	Negative. "
27	<i>Cleome rupestris</i>	Heifer 3876	81 days	11 lb. 2 oz.	Negative.
28	<i>Cluytia</i> (spec. ?).....	Heifer 3878 " 3879	86 days 24 "	27 lb. 1 oz. 6 lb. 13 oz.	Negative. "
29	<i>Convolvulus sagittatus</i>	Heifer 3876	78 days	26 lb. 13 oz.	Negative.
30	<i>Convolvulus ornatus</i>	Heifer 3879 " 2571	94 days 15 "	35 lb. 4 oz. 5 lb. 13 oz.	Negative. "
31	<i>Commelina</i> (spec. ?).....	Heifer 3876 " 3877 " 2724 " 3874	94 days 15 " 60 " 36 "	49 lb. 4 oz. 8 lb. 8 oz. 20 lb. 15 oz. 19 lb. 13 oz.	Negative. " " "
32	<i>Convolvulus</i> (spec. ?).....	Heifer 3875 " 3878 " 2724 " 3874	79 days 78 " 61 " 51 "	38 lb. 6 oz. 30 lb. 4 oz. 14 lb. 5 oz. 18 lb. 10 oz.	Negative. " " "
33	<i>Crassula nodulosa</i>	Heifer 3601	93 days	42 lb. 3 oz.	Negative.
34	<i>Crotalaria spinosa</i>	Heifer 3082	40 days	2 lb. 15 oz.	Negative.
35	<i>Cruciferae</i> (genus and spec.?)	Heifer 3880	78 days	25 lb. 14 oz.	Negative.
36	<i>Cucumis myriocarpus</i>	Heifer 3082	99 days	42 lb. 15 oz.	Negative.
37	<i>Cucurbitaceae</i> (genus and spec.?)	Heifer 3611 " 3383	99 days 8 "	38 lb. 4 oz. 3 lb. 1 oz.	Negative. "
38	<i>Cyathula globifera</i>	Heifer 3876	78 days	24 lb. 9 oz.	Negative.
39	<i>Cyperaceae</i> (genus and spec. ?)	Heifer 3397	75 days	12 lb. 3½ oz.	Negative.
40	<i>Deverra Burchellii</i>	Heifer 3082 " 2724	35 days 63 "	15 lb. 12 oz. 12 lb. 9 oz.	Negative. "
41	<i>Dianthus</i> (spec. ?).....	Heifer 3878 " 3879	99 days 8 "	20 lb. 1 oz. 1 lb. 3 oz.	Negative. "
42	<i>Dicoma capensis</i>	Heifer 3874 " 3875	99 days 8 "	25 lb. 14 oz. 2 lb. 14 oz.	Negative. "
43	<i>Dyschoriste</i>	Heifer 3385 " 3604	82 days 24 "	14 lb. 11 oz. 2 lb. 11 oz.	Negative. "
44	<i>Elephantorrhiza Burchellii</i> ..	Heifer 3385	95 days	31 lb. 6 oz.	Negative.
45	<i>Epaltes gariepina</i>	Heifer 3327	104 days	39 lb. 9 oz.	Negative.
46	<i>Euphorbia sanguineus</i>	Heifer 3877 " 3878	109 days 8 "	33 lb. 1 oz. 4 lb. 3 oz.	Negative. "

TABLE NO. II—(continued).
Plant Feeding Experiments—(continued).

No.	Name or No. of Plant.	No. of Animal.	Total Period of Feeding.	Total Quantity Plant fed.	Result.
47	<i>Felicia fascicularis</i>	Heifer 3601 " 3327	99 days 8 "	26 lb. 11 oz. 3 lb. 0 oz.	Negative. "
48	<i>Gazania longifolia</i>	Heifer 3327 " 3397	94 days 15 "	28 lb. 3 oz. 6 lb. 12 oz.	Negative. "
49	<i>Geigeria zeyheri</i>	Heifer 3875 " 3876	99 days 8 "	46 lb. 3 oz. 4 lb. 10 oz.	Negative. "
50	<i>Geigeria pectidea</i>	Heifer 3879 " 2571	99 days 8 "	45 lb. 13 oz. 4 lb. 12 oz.	Negative. "
51	<i>Giesekia pharnaceoides</i>	Heifer 3397 " 3383	78 days 75 "	18 lb. 12 oz. 21 lb. 11 oz.	Negative. "
52	<i>Helichrysum caespititium</i>	Heifer 3874 " 3875 " 3371	88 days 24 " 99 "	22 lb. 1 oz. 11 lb. 8 oz. 25 lb. 7 oz.	Negative. " "
53	<i>Helichrysum argyrosphaerum</i>	Heifer 3371 " 3611	99 days 8 "	19 lb. 11 oz. 1 lb. 10 oz.	Negative. "
54	<i>Hermannia</i> (spec. ?).....	Heifer 3611	99 days	19 lb. 6 oz.	Negative.
55	<i>Helichrysum</i> (spec. ?).....	Heifer 2571 " 3402 " 3601	75 days 98 " 8 "	11 lb. 12½ oz. 33 lb. 10 oz. 3 lb. 1 oz.	Negative. " "
56	<i>Hermannia candicans</i>	Heifer 3371	78 days	12 lb. 4 oz.	Negative.
57	<i>Hermannia</i> (spec. ?).....	Heifer 3877 " 3878	94 days 15 "	38 lb. 6½ oz. 6 lb. 14 oz.	Negative. "
58	<i>Hermannia</i> (<i>Mahernia cocco-carpa</i>)	Heifer 2571 " 3880	86 days 24 "	25 lb. 10 oz. 5 lb. 7 oz.	Negative. "
59	<i>Hermbstaedtia elegans</i>	Heifer 3880 " 3402	86 days 24 "	27 lb. 7 oz. 9 lb. 12 oz.	Negative. "
60	<i>Hibiscus atromarginatus</i>	Heifer 3874	104 days	12 lb. 1½ oz.	Negative.
61	<i>Hibiscus aethiopicus</i>	Heifer 2571	81 days	9 lb. 5 oz.	Negative.
62	<i>Hypoxis costata</i>	Heifer 3876	107 days	43 lb. 14 oz.	Negative.
63	<i>Indigofera</i> (spec. ?).....	Heifer 3385	75 days	22 lb. 11 oz.	Negative.
64	<i>Indigofera filipes</i>	Heifer 3880	81 days	11 lb. 1 oz.	Negative.
65	<i>Indigofera rhytidocarpa</i>	Heifer 3879	75 days	18 lb. 11 oz.	Negative.
66	<i>Indigofera hiliaris</i>	Heifer 3877	78 days	25 lb. 13 oz.	Negative.
67	<i>Indigofera hololeuca</i>	Heifer 3383 " 3082	36 days 59 "	14 lb. 6 oz. 18 lb. 4 oz.	Negative. "
68	<i>Indigofera patens</i>	Heifer 3082	99 days	29 lb. 2 oz.	Negative.
69	<i>Ipomaea angustisecta</i>	Heifer 3875	74 days	6 lb. 3¼ oz.	Negative.

TABLE II—(continued).
Plant Feeding Experiments—(continued).

No.	Name or No. of Plant.	No. of Animal.	Total Period of Feeding.	Total Quantity Plant fed.	Result.
70	<i>Ipomaea</i> (spec. ?).....	Heifer 3880 ,, 3402	94 days 15 „	42 lb. 4 oz. 5 lb. 9 oz.	Negative. ..
71	<i>Kedrostis punctulata</i>	Heifer 3604	104 days	30 lb. 3 oz.	Negative.
72	<i>Lasiocarys capensis</i>	Heifer 3874 ,, 3875 ,, 2724	15 days 37 „ 45 „	6 lb. 11 oz. 19 lb. 14 oz. 9 lb. 5 oz.	Negative.
73	<i>Lasiosiphon polycephalus</i>	Heifer 2571 ,, 3880	99 days 8 „	34 lb. 8 oz. 3 lb. 1 oz.	Negative. ..
74	<i>Lessertia</i> (spec. ?).....	Heifer 3874	79 days	10 lb. 0 oz.	Negative.
75	<i>Lippia asperifolia</i>	Heifer 3604	99 days	18 lb. 10 oz.	Negative.
76	<i>Listia heterophylla</i>	Heifer 3327	78 days	27 lb. 15 oz.	Negative.
77	<i>Lithospermum arvense</i>	Heifer 3397 ,, 3385	94 days 14 „	30 lb. 14 oz. 3 lb. 0 oz.	Negative. ..
78	<i>Lobelia leptocarpa</i>	Heifer 2571 ,, 3402 ,, 3601	78 days 86 „ 24 „	15 lb. 15 oz. 33 lb. 6 oz. 9 lb. 13 oz.	Negative.
79	<i>Lotononis lenticula</i>	Heifer 3327	93 days	25 lb. 3 oz.	Negative.
80	<i>Malva</i> (spec. ?).....	Heifer 3371 ,, 3611	86 days 24 „	29 lb. 14 oz. 9 lb. 3 oz.	Negative. ..
81	<i>Melolobium</i> (spec. ?).....	Heifer 3874 ,, 3875	76 days 36 „	10 lb. 10 oz. 5 lb. 5 oz.	Negative. ..
82	<i>Menodora africana</i>	Heifer 3397	104 days	34 lb. 15 oz.	Negative.
83	<i>Mesembrianthemum dentata</i> .	Heifer 3601 ,, 3327	86 days 25 „	30 lb. 9 oz. 9 lb. 1 oz.	Negative. ..
84	<i>Microtea Burchellii</i>	Heifer 3397 ,, 3385	94 days 15 „	34 lb. 4 oz. 6 lb. 12 oz.	Negative. ..
85	<i>Monsonia biflora</i>	Heifer 3875 ,, 3879	104 days 27 „	43 lb. 2 oz. 0 lb. 9½ oz.	Negative. ..
86	<i>Nerrine lucida</i>	Heifer 3875 ,, 3876	44 days 36 „	18 lb. 8 oz. 18 lb. 10 oz.	Negative. ..
87	<i>Nidorella solidaginea</i>	Heifer 3601	104 days	41 lb. 11 oz.	Negative.
88	<i>Oldenlandia</i> (spec. ?).....	Heifer 3875 ,, 3876	40 days 15 „	14 lb. 1 oz. 6 lb. 6 oz.	Negative. ..
89	<i>Oldenlandia stricta</i>	Heifer 3402	75 days	11 lb. 9 oz.	Negative.
90	<i>Oxalis compressa</i>	Heifer 3879	81 days	9 lb. 8½ oz.	Negative.
91	<i>Pegolettia polygalaeifolia</i>	Heifer 3880	104 days	35 lb. 7 oz.	Negative.
92	<i>Pentzia virgata</i>	Heifer 3876 ,, 3877 ,, 3397 ,, 3385 ,, 3383 ,, 3082	86 days 25 „ 86 „ 24 „ 99 „ 8 „	33 lb. 8 oz. 13 lb. 4 oz. 35 lb. 9 oz. 9 lb. 10 oz. 28 lb. 4 oz. 3 lb. 14 oz.	Negative.

TABLE NO. II—(continued)
Plant Feeding Experiments—(continued).

No.	Name or No. of Plant.	No. of Animal.	Total Period of Feeding.	Total Quantity Plant fed.	Result.
93	<i>Pharnaceum verrucosum</i>	Heifer 3327	78 days	19 lb. 7½ oz.	Negative.
94	<i>Pollichia campestris</i>	Heifer 3611 „ 3383	94 days 15 „	42 lb. 7 oz. 6 lb. 14 oz.	Negative. „
95	<i>Portulaca pilosa</i>	Heifer 3880 „ 3402	99 days 8 „	32 lb. 6 oz. 1 lb. 14 oz.	Negative. „
96	<i>Portulaca oleracea</i>	Heifer 3611	78 days	35 lb. 14 oz.	Negative.
97	<i>Psoralea obtusifolia</i>	Heifer 3385 „ 3604	99 days 8 „	32 lb. 8 oz. 3 lb. 2 oz.	Negative. „
98	<i>Pterodiscus</i> (spec. ?).....	Heifer 3611	104 days	60 lb. 0 oz.	Negative.
99	<i>Rhynchosia adenoides</i>	Heifer 3875	79 days	33 lb. 2½ oz.	Negative.
100	<i>Rhynchosia nervosa</i>	Heifer 3383 „ 3082	86 days 24 „	23 lb. 2 oz. 7 lb. 11 oz.	Negative. „
101	<i>Salvia runcinata</i>	Heifer 3383	75 days	23 lb. 8 oz.	Negative.
102	<i>Scilla rigidifolia</i>	Heifer 3604	75 days	18 lb. 7 oz.	Negative.
103	<i>Scilla</i> (spec. ?).....	Heifer 3082 „ 2724	51 days 8 „	20 lb. 14 oz. 4 lb. 0 oz.	Negative. „
104	<i>Senecio Burchellii</i>	Heifer 3874 „ 2724	27 days 36 „	2 lb. 3 oz. 3 lb. 11 oz.	Negative. „
105	<i>Senebiera integrifolia</i>	Heifer 3383	104 days	26 lb. 6 oz.	Negative.
106	<i>Sericorema remotiflora</i>	Heifer 3402	78 days	24 lb. 15 oz.	Negative.
107	<i>Sesbania punctata</i>	Heifer 3604	80 days	29 lb. 2 oz.	Negative.
108	<i>Sesamum capensis</i>	Heifer 3611	75 days	32 lb. 10 oz.	Negative.
109	<i>Sisymbrium</i> (spec. ?).....	Heifer 3878 „ 3879	94 days 20 „	41 lb. 4 oz. 7 lb. 11 oz.	Negative. „
110	<i>Sisymbrium</i> (spec. ?).....	Heifer 3879 „ 2571	86 days 24 „	28 lb. 7 oz. 6 lb. 2 oz.	Negative „
111	<i>Stachys spathulata</i>	Heifer 3877 „ 3878	86 days 24 „	37 lb. 10 oz. 10 lb. 1 oz.	Negative. „
112	<i>Stoebe cinerea</i>	Heifer 2724	42 days	6 lb. 12 oz.	Negative.
113	<i>Stapelia</i> (spec. ?).....	Heifer 3371	75 days	24 lb. 11 oz.	Negative.
114	<i>Sutera atropurpurea</i>	Heifer 3877	104 „	36 lb. 9 oz.	Negative.
115	<i>Sutera aurantiaca</i>	Heifer 3385	104 days	13 lb. 10 oz.	Negative.
116	<i>Sutera caerulea</i>	Heifer 3604 „ 2724	36 days 61 „	10 lb. 1 oz. 13 lb. 1 oz.	Negative. „
117	<i>Tephrosia capensis</i>	Heifer 3604 „ 3371	94 days 15 „	21 lb. 5 oz. 4 lb. 3 oz.	Negative. „

TABLE NO. II—(continued).

Plant Feeding Experiments—(continued).

No.	Name or No. of Plant.	No. of Animal.	Total Period of Feeding.	Total Quantity Plant fed.	Result.
118	Thesium Burkei.....	Heifer 3878	78 days	29 lb. 6 oz.	Negative.
119	Thesium (spec. ?).....	Heifer 3371	78 days	8 lb. 4 oz.	Negative.
120	Trichodesma angustifolium..	Heifer 3327	91 days	38 lb. 11 oz.	Negative.
		„ 3397	8 „	3 lb. 8 oz.	„
121	Vahlia capensis.....	Heifer 2571	94 days	38 lb. 0 oz.	Negative.
		„ 3880	15 „	5 lb. 13 oz.	„
122	Vernonia kraussii.....	Heifer 3402	93 days	36 lb. 0 oz.	Negative.
		„ 3601	16 „	6 lb. 4 oz.	„
123	Vigna Burchellii.....	Heifer 3402	93 days	47 lb. 0 oz.	Negative.
124	Walafrida densiflora.....	Heifer 3397	78 days	27 lb. 8 oz.	Negative.
125	82.....	Heifer 3879	104 days	38 lb. 8 oz.	Negative.
126	83.....	Heifer 2571	104 days	35 lb. 4 oz.	Negative.
127	86.....	Heifer 3383	75 days	21 lb. 15 oz.	Negative.
128	131.....	Heifer 3383	99 days	16 lb. 1½ oz.	Negative.
129	158.....	Heifer 3371	104 days	41 lb. 12 oz.	Negative.
130	175.....	Heifer 3402	104 days	17 lb. 15 oz.	Negative.
131	180.....	Heifer 3879	78 days	28 lb. 7 oz.	Negative.
132	182.....	Heifer 3877	78 days	25 lb. 6 oz.	Negative.
133	183.....	Heifer 2571	78 days	30 lb. 14 oz.	Negative.
134	196.....	Heifer 3601	78 days	7 lb. 11 oz.	Negative.
135	199.....	Heifer 3601	78 days	14 lb. 8½ oz.	Negative.
136	204.....	Heifer 3385	78 days	10 lb. 0½ oz.	Negative.
137	207.....	Heifer 3604	80 days	6 lb. 15 oz.	Negative.
138	212.....	Heifer 3878	40 days	2 lb. 1½ oz.	Negative.
139	225.....	Heifer 3371	35 days	4 lb. 15 oz.	Negative.
		„ 2724	61 „	9 lb. 6 oz.	„
140	227.....	Heifer 2724	51 days	5 lb. 4 oz.	Negative.
		„ 3874	45 „	6 lb. 3 oz.	„

The results were entirely negative. Notwithstanding the fact that several plants included in these feeding tests were supposed to be poisonous plants, no ill effects were ever noticed amongst any of the experimental animals.

Remarks.—Contrary to our previous experience, as reported on by Theiler, Green, and Viljoen (l.c.1.), we were now able to keep the experimental animals in quite good condition by feeding them on grasses collected from the veld. In previous experiments the grasses were cut with sickles, so that in this way practically only the coarser and more innutritious stems were brought to the stables. During natural grazing these coarse stems are not eaten by cattle; they confine themselves to the succulent, nutritious leaves growing nearer the surface of the ground. Hence, for the new experiments, the grass was always collected by pulling it out root and all. By feeding grasses collected in this manner the animals remained in good condition throughout the experiment, thus proving the high feeding value of the ordinary veld grass growing on lamziekte areas.

Experiment No. 13.—In quite a number of autopsies on animals that have died from Lamziekte, numerous seeds, which were identified as coming from *Salvia rugosa*, were found in the fourth stomach, and hence it was thought necessary to carry out special tests with this plant. Six animals were drafted into this experiment, each receiving daily 1 lb. of this plant. The feeding was continued at first for one month—from 1st March, 1916, to 1st April, 1916—and later on for a much longer period, from 14th June, 1916, to 26th November, 1916. As a subsistence ration the animals received grasses collected daily from the veld. No symptoms of Lamziekte nor any ill effects were ever noticed amongst the experimental animals.

Experiment No. 14.—Mixed grasses collected daily from the veld were fed to nine animals which were kept in the stable for the purpose. The feeding was continued from 7th September, 1915, to 27th October, 1915, and again from 22nd April, 1916, to 26th November, 1916, the animals receiving as much grass as they could eat. Only a very small supplementary ration, amounting to $1\frac{1}{2}$ lb. bran and $\frac{1}{2}$ lb. bean-meal each per day, was allowed these animals. The main object of the experiment was to find out whether Lamziekte could be produced in animals which were stabled constantly, and which had no access to the veld, by feeding them on mixed veld grasses collected fresh from a lamziekte area. The results were entirely negative.

Remarks.—The grasses were collected in the manner described in Experiment No. 12, and right through the experiment the animals not only maintained their condition, but some actually gained in weight.

Experiment No. 15.—In Experiment No. 1 reference was made to the discovery of a grass (*Chrysopogon serrulatus*) which was specially sought after by cattle, and which was found to be heavily infected with a parasitic fungus (*Phyllachora*). This experiment was commenced on 27th October, 1915, six animals being fed daily on large quantities of *Chrysopogon serrulatus* for a period of six months. At the commencement the grass was badly infected with fungus, but towards the latter half of the experiment the fungus had almost disappeared. The object was to find out whether lamziekte would be produced by feeding animals on the infected grass. The results were entirely negative.

Remarks.—These animals received no additional rations, and towards the conclusion of the experiment had actually gained a great deal in condition, which proves the high feeding value of this grass.

Experiment No. 16.—During the course of the grazing down experiments, described elsewhere, it was found that paddock "C" was very badly "infected" with Lamziekte, and hence it was considered advisable to carry out feeding tests with grasses growing in this paddock. On 3rd July, 1916, four animals were drafted into this experiment, receiving a daily allowance of grasses collected from paddock "C." This grass consisted of only small tufts, and hence it was necessary to allow the animals a supplementary ration consisting of grasses collected from other parts of the farm. The results were entirely negative, although the experiment was continued for nearly five months—until 26th November, 1916.

Experiment No. 17.—The object of this experiment was to find out whether Lamziekte would be produced by feeding animals on certain species of grasses collected from lamziekte veld, and at the same time to find out the nutritive values of the different grasses. On 27th February, 1917, nineteen animals were drafted into this experiment, which was continued for nearly four months—until 8th June, 1917. As there was not sufficient stabling accommodation for a larger number of animals, and as some of the grasses were known to be very innutritious at a certain stage in their growth, each animal received more than one species of grass. In Table No. III the details of the experiment are explained.

TABLE NO. III.
Grass Feeding Experiments.

No.	Name or No. of Grass.	No. of Animal.	Total Period of Feeding.	Result.
1	<i>Antheophora pubescens</i>	Heifer 3874	112 days	Negative.
2	<i>Aristida uniphumis</i>	Heifer 3877 " 3082	112 days 112 "	Negative. "
3	<i>Aristida burkei</i>	Heifer 3879 " 3397 " 2724	112 days 112 " 112 "	Negative. " "
4	<i>Aristida congesta</i>	Heifer 3871	112 days	Negative.
5	<i>Aristida spec.</i>	Heifer 3402	112 days	Negative.
6	<i>Chloris petrea</i>	Heifer 3876	112 days	Negative.
7	<i>Crossotropis grandiglumis</i>	Heifer 3327	112 days	Negative.
8	<i>Chrysopogon serrulatus</i>	Heifer 3601 " 3371	112 days 112 "	Negative. "
9	<i>Cymbopogon plurinodis</i>	Heifer 3874 " 3082	112 days 112 "	Negative. "
10	<i>Digitaria eriantha</i>	Heifer 3879 " 3327	112 days 112 "	Negative. "
11	<i>Elionurus argenteus</i>	Heifer 3611	112 days	Negative.

TABLE NO. III—(continued).

Grass Feeding Experiments—(continued).

No.	Name or No. of Grass.	No. of Animal.	Total Period of Feeding.	Result.
12	<i>Eragrostis superba</i>	Heifer 3875	112 days	Negative.
		„ 3385	112 „	„
13	<i>Eragrostis nebulosa</i>	Heifer 3871	112 days	Negative.
14	<i>Eragrostis spec.</i>	Heifer 3601	112 days	Negative.
15	<i>Eragrostis gummiflua</i>	Heifer 3371	112 days	Negative.
16	<i>Erianthus (spec. ?)</i>	Heifer 3385	112 days	Negative.
17	<i>Fingerhuthia africana</i>	Heifer 3878	112 days	Negative.
		„ 3611	112 „	„
18	<i>Heteropogon contortus</i>	Heifer 3402	112 days	Negative.
		„ 3397	112 „	„
		„ 3383	112 „	„
19	<i>Panicum repens</i>	Heifer 3877	112 days	Negative.
		„ 3880	112 „	„
20	<i>Pogonarthria falcata</i>	Heifer 3878	112 days	Negative.
21	<i>Sporobolus fimbriatus</i>	Heifer 3876	112 days	Negative.
		„ 2724	112 „	„
22	<i>Schmidtia bulbosa</i>	Heifer 3880	112 days	Negative.
23	<i>Themeda Forskalii</i>	Heifer 3875	112 days	Negative.
		„ 3604	112 „	„
24	<i>Tragus spec.</i>	Heifer 3383	112 days	Negative.
25	No. 00.....	Heifer 3604	112 days	Negative.

It will be seen that each animal received two species of grasses every day, and in selecting the grasses for each animal a grass of rare occurrence, as a rule, had to be combined with one growing more abundantly, thus making certain that sufficient grasses for the needs of each animal could be collected every day. No records were kept of the actual quantities fed, but each animal received as much grass as it could eat, without wasting. As far as Lamziekte is concerned, the results were entirely negative. As regards the feeding value of the different grasses, all animals maintained their condition until the last month of the experiment, when the grasses became dry and fibrous, but even at this stage the loss in condition was not very noticeable, with the exception of cow No. 3871, which had been receiving *Aristida congesta* and *Eragrostis nebulosa*. *Aristida congesta*, when mature, is very fibrous, and on account of its prickly seeds is very unpalatable. *Eragrostis nebulosa*, on the other hand, is a very palatable grass, but is much rarer and more difficult to collect, the result being that the

bulk of grass given to this animal consisted of *Aristida*. Loss in condition of this cow was, therefore, quite to be expected.

Experiment No. 18.—This experiment was carried out with the object of finding out whether the grass *Chloris petrea*, which has been found to contain cyanophoric glucoside would, when fed to animals, produce symptoms of Lamziekte or of any other diseased condition. Guignard's simple vest-pocket test for prussic acid in plants was applied to a large number of grasses growing on the lamziekte farm, and so far positive results have been obtained in the case of *Chloris petrea* only. Particulars of the test were supplied to the writer by Dr. H. H. Green, Biochemist, who explained the action of the test as follows:—"The test depends upon the fact that most plants containing a cyanophoric glucoside also contain an hydrolytic enzyme, which liberates hydrocyanic acid from the glucoside during autolysis. This hydrocyanic acid diffuses into the atmosphere of the tube, and colours the paper (picric). Chloroforming the leaf destroys the selective properties of the cellular membranes, and causes alteration in the concentration of the fluids within the leaf, so allowing glucoside and enzyme to come into contact with one another in the sap." With *Chloris petrea* the reaction is always a very strong one, the picric paper being coloured a dark red, so that a large amount of glucoside must be present in the plant. The test has also been applied to samples of *Chloris petrea* growing in the Pretoria District on farms where Lamziekte is unknown, but so far the results have always been negative. On 9th February, 1918, four head of cattle were placed in this experiment, and these animals have been fed solely on *Chloris petrea*, large quantities being consumed every day. Up to the time of writing, after two months of feeding on this grass, no symptoms of any disease have been noticed in the experimental animals. No loss in condition can be noticed, showing that the grass has a high feeding value. The experiment is being continued with both sheep and cattle.

DISCUSSION ON PLANT FEEDING EXPERIMENTS.

As far as Lamziekte is concerned, it must be admitted at once that we have again failed to produce the disease by feeding animals on plants growing on a lamziekte area. The possibility exists—and it would be difficult to overcome—that the toxin is a very unstable one and is likely to disappear before the plants collected for the feeding tests reach the stable. Moreover, it is a well-known fact that Lamziekte at times makes its appearance only in some camps, and possibly only on certain portions of such camps. It is also well known that the disease becomes prevalent only at certain times of the year, and hence one must bear in mind these facts, which could easily explain the negative results obtained from the plant feeding tests. As regards the plant poison, one must also remember the possibility of a combination of plants being necessary for the development of the poison. No definite conclusions could, therefore, be drawn from these negative results. From the purely dietetic point of view the results of these feeding experiments have proved interesting. In a previous paper we, Theiler, Green, and Viljoen (*l.c.*, p. 257), made the following statement regarding grass cut for feeding experiments on Armoedsvlakte:—"The fact that hay made from grass cut at random from lamziekte areas should often prove so innutritious, while the cattle grazing

naturally over the areas themselves generally contrive to keep in fair condition, and to complete the cycle of their being in healthy fashion, except where they specifically contract Lamziekte, is itself of some interest." This statement has been modified by the results of the more recent experiments, in which the animals were kept in excellent condition by feeding them on grasses collected at random from lamziekte veld. As explained in the remarks on the results of Experiment No. 12, the method of collecting the grasses from the veld has a great deal to do with the nutritive value of the hay. In the old experiments the grasses were cut several inches above the ground, thus obtaining for feeding only the coarse and innutritious parts of the plants. In the new experiments the whole plant was taken, so that the succulent and nutritious leaves, which in this part of the country are nearer the surface of the ground, are included in the grasses used in the feeding tests. It may be safely concluded that by feeding stabled animals on fresh grasses collected from the veld every day they can be kept in quite as good condition as animals grazing naturally over the same areas. The results of these experiments also afford strong evidence against the deficiency theories which have been advanced to explain the cause of Lamziekte. Some animals had been stabled for several months, during which their sole ration consisted of grasses and other plants collected from the lamziekte veld, and yet no symptoms of the disease ever developed.

EXPERIMENTS TO TEST SOIL INFECTION THEORIES.

The theory has been advanced that the causal factor of Lamziekte was connected with the soil, and was in the nature of a bacterium or a fungus growing on or in the soil. Although all experimental evidence is against a bacterial origin as far as Lamziekte is concerned, it was thought advisable to carry out some experiments to test this theory.

Experiment No. 19.—The object of this experiment was to find out whether animals receiving in their drinking water soil taken from the badly "infected" paddocks would show a heavier mortality from Lamziekte than animals in other experiments. The experiment was commenced on 6th July, 1916, with forty head of cattle, and was continued until 3rd September, 1917, i.e. for more than a year. Soil from different parts of paddock "C" was collected every day, passed through a rather coarse sieve to keep back all vegetation, stirred in the drinking water contained in troughs to which these animals had access. To ensure that a large amount of soil was ingested by the animals, the water was stirred while the animals were drinking, so as to prevent the soil from sinking to the bottom of the troughs. Some of the animals were also utilized in other experiments, but all of them had access to natural veld grazing.

Cases of Lamziekte.—(1) Heifer No. 3606 died three months after it was placed in this experiment. This animal had recovered from an attack of Lamziekte three months previously. (2) Cow No. 2170 showed symptoms on 26th November, 1916, over four months after it was put in the experiment, and died a few days later. (3) Heifer No. 2347 showed symptoms on 27th November, 1916, and died three days later.

Remarks.—The percentage of cases of Lamziekte in this experiment was quite small, no larger than that amongst animals in other experiments or amongst the controls. The results of this experiment are, therefore, against the theory that the causal factor of Lamziekte is connected with the soil on lamziekte areas.

Experiment No. 20.—A similar experiment was carried out on the farm Onderstepoort, where Lamziekte is unknown, soil being obtained from the "infected" paddocks on Armoedsvlakte. The experiment was commenced on 22nd August, 1916, and continued for more than a year, until 28th August, 1917, the average number of animals in the experiment being forty. In addition to the soil received in their drinking water, these animals also had access to a lick consisting of one part common salt, one bone-meal, and two parts soil. The results were entirely negative, no case of Lamziekte occurring.

Experiment No. 21.—To test the soil infection theory still further this experiment was carried out. From sub-division 3, paddock "B," a well-known lamziekte area, all vegetation was removed, and on 25th August, 1916, when the area was quite free from vegetation, twenty head of cattle were placed in this sub-division. The animals were fed on a maintenance ration, consisting of foodstuffs from a non-lamziekte area, and were allowed to roam about as much as they liked in search of natural grazing. By allowing these animals to roam about and lick the soil, and by placing their daily rations on the ground, it was hoped that a fair amount of soil would be ingested. With the exception of a few cows suckling small calves, which had to be taken out of the experiment on account of loss of condition, all animals remained in good health. The experiment has now been continued for over 1½ years, and up to the time of writing no cases of Lamziekte have occurred.

Remarks.—The possibility exists that these animals did not ingest a great deal of soil, but if the cause of Lamziekte is connected with a bacterial infection of the soil there should have been no need for the ingestion of a large amount of soil to produce the disease.

Experiment No. 22.—The object of this experiment was to find out whether Lamziekte could be conveyed in the soil from a lamziekte to a non-lamziekte farm. For this purpose 150 tons of soil were sent from different camps on Armoedsvlakte to Onderstepoort, where the soil was spread evenly over a previously prepared area. On this imported soil vegetation was allowed to grow for grazing purposes. At different periods of the year, April, 1916, to April, 1917, cattle were allowed to graze over this area, twenty-one head of cattle having so far had access to this veld. The results have been entirely negative, suggesting that Lamziekte could not be conveyed in the soil from a lamziekte to a non-lamziekte farm, and thus affording evidence against the soil infection theory.

DISCUSSION OF RESULTS FROM "SOIL" EXPERIMENTS.

In considering the results obtained from the four experiments just described, one cannot help but come to the conclusion that the causal factor of Lamziekte has no connection with soil "infection." In Experiment No. 19 forty animals received daily in their drinking water for more than a year soil from bad lamziekte camps, so that the

amount of soil ingested by these animals must have been enormous, and yet the mortality from Lamziekte amongst these animals was no heavier than that amongst other experimental batches. In Experiment No. 20, which was carried out on similar lines on a non-lamziekte farm, the results were even more conclusive, not a single case of Lamziekte occurring amongst a batch of forty animals.

ALTERNATE FEEDING AND GRAZING EXPERIMENTS.

Elsewhere in this report further evidence is brought forward that extra feeding does not influence the incidence of Lamziekte, providing the animals receiving the extra feeding have access to lamziekte veld. The main object of the experiments to be described here was to find out whether there would be any difference in the incidence of the disease when animals were kraaled and given a well-balanced ration for a certain period, and then allowed to run on the veld without any additional rations for another period. By varying the periods of kraaling and grazing it was hoped to arrive at the period of "incubation," or latent period of the disease.

Experiment No. 23.—On 22nd October, 1916, forty head of cattle were drafted into this experiment and kept in the kraal constantly for one week, after which they were allowed to run in the veld for one month. During the period of kraaling the animals received a mixed ration, consisting of 3 lb. of crushed mealies, 2 lb. bran, 7 lb. lucerne hay, and 7 lb. veld hay per animal per day. The experiment was continued for nearly ten months, until 14th August, 1917. The following cases of Lamziekte occurred:—(1) Heifer No. 3554 showed symptoms on 15th November, 1916, and died the same day, or three weeks after it was put in the kraal, and seventeen days after it left the kraal. (2) Ox No. 2893 showed symptoms on 26th November, 1916, and died the next day, or twenty-eight days after leaving the kraal, and thirty-five days after it was put in the kraal. (3) Cow No. 2579 showed symptoms on 2nd December, 1916, and died the following day, or on the fourth day after it returned to the kraal for the second time. (4) Cow No. 643 showed symptoms on 16th December, 1916, and died two days later; symptoms appeared, therefore, ten days after the animal left the kraal, or seventeen days after it was put in the kraal for the second time. (5) Cow No. 3163 was noticed ill on the morning of 9th January, 1917, and died the same afternoon, i.e. on the third day after arriving back in the kraal. (6) Heifer No. 3304 showed symptoms on 9th January, 1917, and died the same day, i.e. on the third day after it was returned to the kraal.

Remarks.—The mortality amongst this batch of animals was higher than that amongst other experimental animals for the same period, proving that feeding in itself does not decrease the mortality. On the other hand, the complete change of diet from veld grazing to kraal feeding seems to increase the mortality. In this connection it is interesting to note that no fewer than three out of the six fatal cases showed symptoms a few days after the change from veld to stable diet.

Experiment No. 24.—This experiment was conducted on similar lines to No. 23, excepting that the periods of kraaling and grazing were different. Forty animals were drafted into the experiment on 21st February, 1917, the period of kraaling being fourteen days, and

the period of grazing also fourteen days. The daily ration was the same as that in the previous experiment. The results were entirely negative, although the experiment was continued for nearly six months, until 14th August, 1917.

Remarks.—During the period of this experiment Lamziekte was practically absent from the farm, so that no definite conclusions could be drawn from the results.

DISCUSSION OF RESULTS.

As already mentioned in the remarks on the results of Experiment No. 23, the periodic change of diet from veld grazing to kraal feeding does not reduce the incidence of Lamziekte, but rather increases the mortality from the disease. As regards the "incubation" or latent period of Lamziekte, nothing definite has been learned from the results of these experiments. Three animals showed symptoms of the disease three to four days after returning to the kraal, so that it may be concluded that symptoms do not necessarily appear immediately after the disease is contracted. The three other animals showed symptoms from the tenth to twenty-eighth days after returning to the veld, but as the period of kraaling was only seven days there is no certainty that the disease was contracted before or after the period of kraaling.

EXPERIMENTS TO TEST FOOD DEFICIENCY THEORIES.

For many years past, and even to the present day, Lamziekte has been considered by some observers to be a "deficiency disease," due to the lack of some essential food constituent in the veld on lamziekte areas. A mineral deficiency, especially as regards phosphates, has been most generally accepted as a possible cause of the disease. No deficiency of any kind can explain all the observations which have been made in connection with Lamziekte, and a mineral deficiency finds no support in the fact that the best conditioned animals are the ones to contract Lamziekte first, and that calves which are born and bred on lamziekte farms develop normally, are not stunted in appearance, and never show any malformation of the bones. In order to give this theory a fair trial, however, the following experiments were carried out:—

Experiment No. 25.—Lucerne and Bran Feeding.—On 14th July, 1915, twenty animals were drafted into this experiment, and fed on a daily ration consisting of 15 lb. lucerne hay and 5 lb. bran for each animal. The quantities were selected with a view of balancing out the phosphorus and calcium in a ration approximating to that of utilization in metabolism. The object of the test was two-fold: (1) To supply a natural ration high enough in quantity and quality to practically cover the grass requirements of the cattle, so that the factor of veld feeding would be eliminated to a large extent, while the factor of veld roaming would be retained. In this way it was hoped to obtain information concerning the infection and the intoxication theories. (2) To supply minerals, more especially calcium and phosphorus, in a natural form in an adequate amount, and in this way to test the mineral deficiency theory and to control the mineral lick experiment, to be described later. Unfortunately, it was soon found that the quantity of the ration was

too large, and that, in fact, the animals did not consume all their rations, which were reduced in consequence to 9 lb. lucerne and 3 lb. bran each per day. The one object of the test, viz., elimination of veld grazing, was, therefore, defeated, but the ration still contained sufficient minerals to make up for the alleged or suspected deficiency in the veld. The animals were allowed to run on the veld constantly, except that they were brought to the kraal in the morning for feeding. Unfortunately, the mistake was made to separate the twenty animals into two batches for grazing, one batch of ten being placed in sub-division 1, paddock "B," for the period 4th July, 1915 to 14th August, 1917. This matter will be referred to again later.

Cases of Lamziekte.—(1) Heifer No. 3606 died on the 24th November, 1916. It was placed in the experiment on 16th August, 1916, so that death took place just after the animal had been in the experiment three months. (2) Cow No. 2170 first showed symptoms on 27th November, 1916, after it had been in the experiment for four and a half months, and died on 4th December, 1916.

Remarks.—As a result of lucerne and bran feeding the animals remained in excellent condition throughout the course of the experiment. Only two cases of Lamziekte occurred, and both these were from the one batch of ten animals; the other batch of ten animals remained for grazing in sub-division 1, paddock "B," where no cases of the disease occurred amongst a total of twenty animals.

Experiment No. 26.—Mineral Lick.—The mineral mixture consisted of the following ingredients:—

Mag. sulph., 15 parts; common salt, 30 parts; fine bone-meal, 30 parts; sodium phosphate, 4 parts; calcium fluoride, 1 part; precipitated chalk, 19 parts; iron sulphate, 1 part. Each of the twenty animals, which were drafted into the experiment on 17th July, 1915, received a daily ration of 8 oz. of the mixture, which was considered sufficient for the requirements of the animal, even if there was a deficiency of minerals in the veld. The object was to supply minerals, more especially calcium and phosphorus, in an adequate amount, and in this way to test the deficiency theory. It was considered that animals receiving a daily ration of 8 oz. of the mixture should not develop Lamziekte, if the theory of a mineral deficiency in lamziekte veld were correct. These animals were also divided into two lots of ten each, but all the animals were allowed to graze in the large camps on the farm. At first the lick was not taken well by the cattle, but by mixing it with a very small quantity of bran the animals were induced to take to it. Soon after that, and right through the course of the experiment, the mixture was readily and even greedily licked by the cattle.

Cases of Lamziekte.—(1) Tollie No. 2862 first showed symptoms on 27th August, 1915, a month and ten days after the experiment was started, but it made a complete recovery. (2) Ox No. 2272 died on 11th December, 1915, nearly five months after the experiment was started. (3) Cow No. 2314 showed symptoms on 29th April, 1916, and made a good recovery. This animal had been in the experiment over nine months. (4) Cow No. 3163 died on 9th January, 1917. It was placed in the experiment on 16th August, 1916, and had, therefore, been in the experiment close on five months.

Remarks.—Within a few months after the experiment was started, an improvement in the condition of the cattle could be noticed, and after that the animals remained in very good condition. It is rather remarkable, but it may be only a coincidence, that two out of the four cases recovered from the disease. It is impossible to say whether this was due to an improved constitution conferred on the animals by the extra supply of minerals. The experiment was discontinued on 14th August, 1917.

Experiment No. 27.—Bone-meal Feeding.—For many years past farmers and others have been supplying a bone-meal lick to their cattle as a preventive against Lamziekte, and the results obtained have varied enormously, some farmers stating that the bone-meal has made no difference to the mortality from Lamziekte, whilst others, who are undoubtedly in the minority, still maintain that the bone-meal lick has preventive properties against the disease. The bone-meal given to the animals is supposed to provide the animals with minerals which are believed to be lacking in the grass growing on lamziekte farms. This experiment was commenced on 14th July, 1915, with twenty animals, each receiving a daily ration of 8 oz. of bone-meal, which can be considered a very liberal allowance when compared with that given by farmers. For the first few days the bone-meal was not taken very readily by the cattle, but as soon as they got accustomed to it they took it not only very readily but even greedily.

Cases of Lamziekte.—(1) Ox No. 2359 showed symptoms on 15th November, 1915, four months after the experiment was started, and died the following day. (2) Heifer No. 2339 showed symptoms on 22nd January, 1917, six months after it was placed in the experiment, and made a good recovery. Heifer No. 2183 showed symptoms of typical Stijfziekte on 17th January, 1917, eighteen months after the commencement of the experiment, and as bone-meal is alleged to be a specific for this disease, this case is all the more interesting.

Remarks.—In this experiment again the animals were divided for grazing into two batches of ten each, and only the one lot, amongst which the two cases occurred, was allowed to run in the large camps, so that the mortality must be reckoned as two out of ten. The other lot of ten animals was placed in sub-division 2, paddock "D," where only one case of Lamziekte occurred amongst another lot of cattle, and there is considerable doubt as to whether this animal did not contract the disease in one of the larger camps. After a few months' feeding on bone-meal the improvement in condition of these animals was very marked, and the good condition was maintained right throughout the course of the test. The experiment was discontinued on 14th August, 1917.

Experiment No. 28.—Bone-meal Feeding.—During the same period as the previous experiment was going on a number of surplus animals was running on the farm, and to these it was decided to give a lick of bone-meal. On 5th September, 1915, when the experiment was commenced, the surplus cattle consisted of about twenty animals, but this number was gradually increased until it reached forty on 24th May, 1916, when the experiment was discontinued. The manner of giving the lick in this case varied slightly from that in the previous experiment; instead of rationing the animals every day, their troughs

were filled with bone-meal once a week, the amount given being equivalent to 8 oz. per animal per day. The lick was well eaten by the cattle, and very often the troughs were emptied after only three or four days.

Cases of Lamziekte.—(1) Cow No. 2313 showed symptoms on 22nd March, 1916, more than six months after feeding on bone-meal was started, and died two days later. (2) Cow No. 3157 died on 22nd April, 1916, four and a half months after it was placed in the experiment. (3) Cow No. 2618 showed symptoms on 1st January, 1916, four months after it was put in the experiment, and died two days later. (4) Heifer No. 2444 died on 7th January, 1916, after four months' feeding on bone-meal. (5) Heifer No. 2416 was noticed ill on 23rd October, 1915, and died two days later.

Remarks.—The average number of animals in this experiment was about thirty, so that the percentage of Lamziekte cases works out at 17 approximately. This may be considered a very high mortality, when it is remembered that the experiment was continued for only about eight and a half months, whereas the other experiment of this series occupied a period of over two years. These animals were running in a camp by themselves, and there is no doubt that this camp was badly "infected" with Lamziekte at that time. Improvement in the condition of these cattle as a result of bone-meal feeding was also very marked.

Experiment No. 29—Antiseptic Lick.—Strictly speaking, this experiment should not fall under the series of tests for the deficiency theories, but as bone-meal was one of the chief ingredients of the lick, and as the experiment was merely a continuation of the last, it would perhaps be advisable to discuss the results here. The same animals were used as in the previous experiment, but, instead of giving a lick consisting of bone-meal only, several other ingredients were added, the lick being made up as follows:—Common salt, 8 oz.; bone-meal, 8 oz.; iron sulphate, 2 drms.; arsenic, 30 grs.; copper sulphate, 1 drm.; creosote, 1 drm.; ginger, 2 drms. These quantities were intended as a single dose for an animal, and were to be given three times weekly. Apart from the bone-meal, therefore, the mixture contained ingredients which had a tonic effect as well as an antiseptic action on the bowels. The latter were given with the object of neutralizing and preventing the absorption of toxins from the bowels. On account of the strong smell and taste of the creosote the animals did not like the lick at first, but after a while they took to it quite well.

Cases of Lamziekte.—(1) Heifer No. 3384, showed symptoms on 15th June, 1916, and died the same day, only three weeks after the experiment was started. This case is interesting, in that it was a Pretoria animal, whence it arrived on 5th September, 1915, and showed symptoms on 16th February, 1916, or about five months after its arrival at Armoedsvlakte. (2) Black heifer showed symptoms on 2nd August, 1916, about two and a half months after the experiment was started, and died two days later. (3) Heifer No. 3602 showed symptoms on 3rd August, 1916, and made a complete recovery. (4) Heifer No. 3493 showed symptoms on 11th August, 1916, and died three days later. (5) Heifer No. 3387 died on 21st August, 1916, after having been in the experiment for three months. (6) Heifer No. 3558 died on 5th September, 1916. (7) Heifer No. 3602 showed symptoms on 9th September, 1916, and died two days later. This heifer is the same as No. 3 above, and died from the second attack of the disease

within five weeks from the first attack in the same experiment. (8) Cow No. 651 showed symptoms on 22nd September, 1916, and died the next day. (9) Heifer No. 3723 was noticed to be ill on 22nd September, 1916, and died the following day. (10) Heifer No. 3645 died on 30th September, 1916. (11) Cow No. 2607 died on 24th November, 1916. (12) Heifer No. 3620 showed symptoms on 4th December, 1916, and made a complete recovery. (13) Heifer No. 3816 showed symptoms on 22nd January, 1917, and died on 26th January, 1917. (14) Heifer No. 3376 died on 4th October, 1916.

Remarks.—Counting No. 3602, which appears twice in the above list, as only one case, it will be seen that the mortality amongst the animals in this experiment has been exceptionally heavy, amounting to 32 per cent., when the average of the number of animals is taken as forty. It is true that these animals received only about half the quantity of bone-meal as was given to animals in the other two bone-meal feeding tests, but even this reduced ration is much more than the quantity which a farmer would allow his animals. That the mixture had a tonic effect was proved by the fact that the animals improved a great deal in condition. These animals were grazing in the same camp as those in the preceding experiment, and, judging by the high mortality, one must conclude that this camp was badly “infected” during the period of the experiments, and that neither the bone-meal nor the antiseptic tonic mixture had any preventive action against the development of Lamziekte.

Experiment No. 30—Controls.—To control the results of these experiments, and more especially those of Experiments Nos. 25, 26, 27, twenty animals were selected to run as controls with the experimental batches. Here again the mistake was made to divide the animals into two sets of ten each, one of which was kept constantly with lots of ten from the experimental batches, while the other remained only with the lot from Experiment No. 26 (mineral mixture) and not with the animals in the other two experiments. Both sets of controls, however, had continual access to the general lamziekte veld.

Cases of Lamziekte.—(1) Heifer 2912 showed symptoms on 16th November, 1915, and died three days later. (2) Cow No. 3001 showed symptoms on 13th December, 1915, and died four days later. This animal passed through a previous attack of the disease on 19th March, 1915. (3) Heifer No. 3181 was noticed ill on 17th January, 1916, and died the following day. (4) Heifer No. 2347 showed symptoms on 27th November, 1916, and died five days later. (5) Ox No. 2445 died on 9th September, 1915. (6) Heifer No. 2893 was noticed ill on 26th November, 1916, and died the next day.

Remarks.—The percentage of cases in this batch works out at 30, whereas the percentage of cases in the other experiments was only 20. The percentage of cases amongst the controls, therefore, appears to be rather high when compared to that amongst animals in the experimental batches, but it must be remembered that in reality in the lucerne and bran and bone-meal feeding tests only ten animals come up for consideration, as the other ten of each batch were grazed in paddocks in which the disease was completely absent. Moreover, in the later bone-meal test (Experiment No. 28) the percentage of cases for only eight and a half months was 17, and in the antiseptic lick (Experiment No. 29) test the percentage of cases was 32, actually higher than that in the controls. Experiment No. 29 may be considered a continuation of Experiment No. 28, and hence, taking the

average of the percentage cases in these two experiments, we have a percentage of 24, which is not far behind that of the controls. One fact, however, was brought out very clearly, and that was that the animals in the feeding experiments were in much better condition than those in the controls.

DISCUSSION OF RESULTS.

In analysing the results obtained from the experiments which were carried out to test the mineral deficiency theories, we have the following cases of Lamziekte amongst the different batches:—

- (1) Lucerne and bran feeding: 2 out of 10, or 20 per cent. in a period of two years.
- (2) Mineral lick: 4 out of 20, or 20 per cent., for a period of two years.
- (3) Bone-meal feeding: 2 out of 10, or 20 per cent., for a period of two years.
- (4) Bone-meal feeding (surplus cattle): 5 out of 30, or 17 per cent., in a period of only eight and a half months.
- (5) Antiseptic tonic lick: 13 out of 40, or 32 per cent., in a period of only fifteen months.
- (6) Control cattle: 6 out of 20, or 30 per cent., for a period of two years.

Unfortunately, owing to economic considerations, both as regards animals and food-stuffs, some animals had to be utilized in more than one experiment, and hence received their grazing on different parts of the farm. It is a well-known fact that the prevalence of Lamziekte on different parts of the farm during the same season may vary enormously, and hence the results of these experiments have been upset to a large extent by the procedure already explained. In the percentage cases given above, we find a difference of 10 per cent. between the control animals and those in other experiments, excepting, however, the antiseptic lick batch, amongst which the percentage of cases was actually higher than amongst the controls. Considering the small number of animals employed in the various experiments, the difference in the percentage of cases is not so very great, and can be easily explained by the fact that the animals in the different batches did not always run together in the same camp. If Lamziekte were due to a mineral deficiency in the veld, no cases of the disease should have occurred amongst the experimental batches, because the quantity of minerals supplied in the rations could reasonably have been expected to make up for any deficiency which might have existed in the veld, especially as some animals had been receiving the mineral rations for quite long periods. The argument has been brought forward that by giving the minerals as a lick the very animals which contracted the disease may not have ingested very much of the mixture. Against this argument, however, stands the fact that after a while the animals consumed their rations not only readily but also very greedily. Moreover, the feeding was always carried out under supervision, a separate feeding trough, as far as possible, being provided for each animal. There is no doubt, however, that all the different rations had a beneficial effect on the condition of the experimental animals, and in the case of the mineral mixture and the antiseptic lick this can be explained by the mineral tonics which these mixtures contained.

In the case of the lucerne and bran feeding test also the improvement in condition of the animals can be explained by the high feeding value of these food-stuffs. The improvement in condition of the animals receiving bone-meal is not so easily understood. It is not conceivable that a great deal of the mineral salts contained in bone-meal could be made available by the digestive system of the animal. To the writer it seems more probable that the beneficial effect derived from the bone-meal is attributable to the tonic effect on the digestive tract, merely in a mechanical way. The strongest evidence which can be brought against the deficiency theories is afforded by the plant and grass feeding experiments, in which the experimental animals were kept in excellent condition by feeding on grasses collected from lamziekte veld. The animals were kept in the stable and fed on these grasses for eight and a half months, and in spite of this long period of feeding on grass, which is alleged to have some sort of deficiency in it, the animals remained in perfect health and never showed any signs of Lamziekte.

Since writing the above, a further very interesting result has been obtained from a bone-meal feeding test which is now being carried out. One out of twenty animals which were drafted into the experiment on 17th December, 1917, showed symptoms of Lamziekte exactly three months later. In this experiment each animal was actually dosed every morning with 8 oz. bone-meal, so that there cannot be the slightest doubt that the animal received the correct amount of bone-meal. During the same three months only two other cases of Lamziekte occurred on the farm, and considering the fact that these two cases occurred out of about 230 head of cattle, it is quite clear that the disease was not prevalent during the three months in question.

EXERCISE EXPERIMENTS.

Oxen have always been held to show only slight susceptibility to Lamziekte, and this has been attributed to the fairly regular exercise to which these animals are subjected, the theory being that the poison or toxin is eliminated from the tissues by the increased metabolism resulting from the muscular exercise. In explaining his "Accumulative Toxin Theory," Theiler states:—"It is generally stated that working oxen, and also cows which are put in the plough, do not contract the disease, whereas oxen and tollies not working are more liable. This fact we can explain. . . . When an ox or a cow is worked such work can only be obtained at the expense of energy which is obtained by the increased oxidation taking place in the muscular system. The increased oxidation also involves the toxin, and which, being of an organic nature, must undergo the process of metabolism, and thereby is destroyed."

Experiment No. 31.—In order to find out whether exercise really prevented the appearance of Lamziekte, as claimed by many observers, it was considered necessary to utilize for this experiment not only oxen, but also cows, heifers, and tollies. Consequently, 38 head of mixed cattle, comprising oxen, cows, heifers, and tollies, in approximately equal numbers, were selected on 14th July, 1915, and drafted into this experiment. The cows, heifers, and tollies were all untrained to the yoke, but the training of these animals was commenced at once, and, as was to be expected, some of the selected animals proved to be unsuitable for the experiment, and had to be replaced by others. The

animals were given fairly severe exercise regularly several hours a day, being used for draught purposes in the wagon, in the plough, or in the "sledge." Owing to the comparatively large number of animals employed in the experiment, it was impossible to exercise the whole batch in one day, but efforts were made to work all the animals at least every other day. Careful daily records were kept of the nature and the amount of exercise received by each animal. The work was continued for four or more hours every day, and there is no doubt that these animals received more exercise than the average farmer would give his oxen. On an average the animals in the experiment remained at about thirty-three, and the mortality from these was nine, a percentage of about 27, which was exceptionally high when compared to other experiments for the same period. These animals received nothing in the nature of supplementary rations, excepting a lick of common salt, to which all the animals on the farm had access. Table No. 4 shows the number of days preceding death on which the fatal cases were exercised, each day representing four or more working hours. Some of the cases received exercise right up to the day when symptoms of the disease developed.

TABLE NO. IV.
Exercise Experiment — Work Table.

Nos. of Animals concerned.	Sex of Animal.	Total Number of Working Days preceding Death.	No. of Days Animal received Exercise.	Percentage of Days Animal exercised to Working Days.
2601	Cow	25	7	28
2396	Tollie	49	25	50
2459	Ox	66	26	40
2270	Ox	75	33	44
2627	Heifer	86	15	19
2263	Ox	113	60	53
2277	Ox	188	98	52
2353	Ox	284	139	49
2427	Ox	361	177	49

In analysing the above figures it will be seen that the majority of fatal cases received exercise on about 50 per cent. of the available days, which means that these animals were worked on about every other day. In the case of the cow and the heifer the percentage of days on which these animals received exercise was much smaller, and this can be accounted for by the unsuitability of these animals for severe exercise, so that on days when extra hard work was required of the cattle, some cows and heifers were left out.

As soon as exercise was found to be a failure as far as the prevention of Lamziekte was concerned, all breeding cattle in the experiment were replaced by oxen, and this is the reason that the majority of fatal cases were oxen. Cows and heifers were considered to be no longer necessary in the experiment, for the simple reason that if exercise did not prevent the disease in oxen it could not be expected to have any preventive effect in the case of the more susceptible cows and heifers.

Remarks.—The results of this experiment may be considered to have proved conclusively that muscular exercise does not have the slightest effect on the incidence of Lamziekte. In fact, the mortality

amongst the exercised animals was as high or higher than that amongst animals in other experiments for the same period. It must be pointed out, however, that these animals were never allowed to graze on any other farm, as would be the case with oxen belonging to farmers. In the latter case the oxen are very often used and allowed to graze, not only on different parts of the same farm, but also on other farms, so that they frequently have a change of veld. It is to this change of veld that most probably the smaller mortality from Lamziekte amongst oxen is due.

TRANSMISSION EXPERIMENTS.

With the object of testing the "infection theory," as applied to Lamziekte, extensive experiments have been carried out by previous workers, with negative results in all cases. The conclusion that Lamziekte is neither infectious nor contagious was, therefore, perfectly justified. In this series of experiments further methods will be described by which attempts were made to transmit the disease from sick and dead animals to healthy animals.

Experiment No. 32.—Blood Transfusion.—The object of this experiment was not so much to test the "infection theory" as to find out whether the disease could not be transmitted in a mechanical way from a sick to a healthy animal by the transfusion of large quantities of blood. Assuming Lamziekte to be caused by a plant toxin, it is reasonable to expect this toxin to be conveyed from the intestines through the blood-stream to the muscles, and that, therefore, in the early stages of an acute attack of the disease there might possibly be a good deal of toxin circulating in the blood-stream. Details of the transfusion experiments are shown in Table No. 5, from which it will be seen that so far ten animals have been used in these experiments, the blood being taken from seven animals suffering from Lamziekte.

TABLE NO. V.
Blood Transfusion Experiment.

Nos. of Animals into which Blood Transfused.	Date of Transfusion.	Nos. of Animals from which Blood Transfused.	Quantity of Blood Transfused.	Result.
3879	15.10.17	3878	3,000 c.c.	19.10.17, Lamziekte.
3877	18.10.17	3879	1,600 c.c.	Negative.
3641	18.10.17	3879	1,500 c.c.	"
3646	27.11.17	Cow (Smollan)	3,500 c.c.	"
3876	27.11.17	"	1,000 c.c.	"
3470	11.12.17	3098	3,500 c.c.	"
3653	13.12.17	2911	2,800 c.c.	"
3642	13.12.17	3222	3,150 c.c.	"
3722	12. 2.18	3649	3,000 c.c.	"
3654	12. 2.18	3649	2,500 c.c.	"

All these animals had been running in lamziekte camps for some time, but on 28th September, 1917 they were put in the stable and kept there constantly, receiving only food-stuffs from an area where Lamziekte was unknown. A few hours every day they were allowed out for exercise, but during this time they were muzzled with wire

muzzles, which had sufficiently small meshes to prevent the entrance of food-stuffs of any kind.

One positive case resulted from these experiments. On 15th October, 1917, heifer No. 3879 received by transfusion three litres of blood from heifer No. 3878, which was suffering from an acute attack of Lamziekte. Nothing untoward happened until the third day after the transfusion, when definite symptoms of Lamziekte developed. The disease ran a typical course, the animal dying the next day; the post-mortem findings were those of Lamziekte, and later on when the muscles were examined microscopically the degenerations usually found in the muscles of Lamziekte animals were present in this case. Blood from this heifer was transfused into two other animals with negative results in both cases; and since then seven further animals have had blood transfused into them from five other cases of Lamziekte, but the results were again entirely negative.

Remarks.—As already mentioned, the experimental animals had access to the general lamziekte veld before these experiments were started, and in the case of the one positive result (heifer No. 3879) the transfusion was carried out only seventeen days after the animal was brought from the veld to the stables. Symptoms of Lamziekte developed three days later, or twenty days after the animal was taken away from the lamziekte veld. Previous observations on Lamziekte have pointed to an "incubation" or latent period in connection with this disease, and in the present case it is extremely likely that the animal suffered from a natural attack of the disease, which was contracted on the veld before the animal was brought to the stable. The transfusion experiments have been repeated over and over again, always with negative results, so that the view expressed above appears to be the correct one.

Experiment No. 33—Muscle Inoculations.—The experiments to be described here were carried out under the supervision of Mr. G. de Kock, Veterinary Research Officer, who was relieving officer during the period the writer was on military duty. To make the report of our investigations into Lamziekte more complete, the writer has been instructed to write up the details of these experiments and to include them in this paper. These experiments were suggested by the results of investigations conducted by Professor Hedinger,* who came to the conclusion that the cause of Lamziekte was connected with the presence of Sarcosporidia and their toxin in the muscles of cattle. The main object of the experiments was, therefore, to see whether it would be possible to transmit the disease by the injection of flesh containing the Sarcosporidia. Animals suffering from the disease were killed after Lamziekte was definitely diagnosed, and their muscles collected in the fresh state. These muscles were then passed through a mincing machine, those minced only once being called "coarse," and those passed through the machine twice being named "fine" muscles. An emulsion was then made with the minced meat in physiological or distilled water, and this emulsion was used for the inoculation of healthy cattle. Details of the experiments are shown in Table No. 6, from which it will be seen that eight fatal cases occurred, all diagnosed by Mr. De Kock as suspected Lamziekte.

* Hedinger, E.: Pathological Investigation into Lamziekte, Department of Agriculture, Union of South Africa, 1915.

TABLE NO. VI.
Muscle Injection Experiment.

No. of Animal.	Previous History.	Date of Inoculation.	Material Used.	Animals from which Material Obtained.	Method of Inoculation.	Treatment received during Period of Experiment.	Result.
2882	Running in field.....	27.10.14 18.11.14	Fine lamziekte muscle..... Fine lamziekte muscle.....	Ox 2505 Heifer 2841	Intrajugularly Subcutaneously	27.10.14, grazing day and night in small Camp C 1.11.14, turned into Camp D 10.11.14, kraaled and given grass and small ration	Died acute Lamziekte (?) 27.11.14.
2345	Control, running day and night in Camp D	27.10.14 18.11.14 19.12.14 22.1.15 17.2.15	Fine lamziekte muscle..... Fine lamziekte muscle..... 20 c.c. fine lamziekte muscle..... Coarse lamziekte muscle..... Coarse lamziekte muscle.....	Ox 2505 Heifer 2841 Bull 3015 Ox 2262 Cow 2604	Intramuscularly Intrajugularly Drenched Subcutaneously Intraperitoneally	27.10.14, grazing day and night in small Camp C 1.11.14, turned into Camp D 10.11.14, kraaled and given grass and small ration	Negative.
2273	Running in Camp D.....	27.10.14 18.11.14	Fine lamziekte muscle..... Fine lamziekte muscle.....	Ox 2505 Heifer 2841	Subcutaneously Intraperitoneally	27.10.17, grazing day and night in small Camp C 1.11.14, turned into Camp D 10.11.14, kraaled and given grass and small ration	Died acute Lamziekte (?) 26.11.14.
2457	Running in Camp D.....	27.10.14 18.11.14 19.12.17	Fine lamziekte muscle..... Fine lamziekte muscle, 2 pints..... 20 c.c. fine lamziekte muscle.....	Ox 2505 Heifer 2841 Bull 3015	Intraperitoneally Drenched Intramuscularly	27.10.14, grazing day and night in small Camp C 1.11.14, turned into Camp D 10.11.14, kraaled and given grass and small ration	Transferred on account of poverty 1.1.15.
3036	Grazing day and night in Camp B	27.10.14 18.11.14	Coarse lamziekte muscle..... Coarse lamziekte muscle.....	Ox 2505 Heifer 2841	Intrajugularly Subcutaneously	27.10.14, grazing day and night in small Camp C 1.11.14, turned into Camp D 10.11.14, kraaled and given hay and small ration	Died per acute Lamziekte (?) 24.11.14.
2700	Grazing day and night in Camp D	27.10.14 18.11.14 19.12.14 22.1.15 17.2.15	Coarse lamziekte muscle..... Coarse lamziekte muscle..... 20 c.c. coarse lamziekte muscle..... 10 c.c. fine lamziekte muscle..... Fine lamziekte muscle.....	Ox 2505 Heifer 2841 Bull 3015 Ox 2262 Cow 2604	Intramuscularly Intrajugularly Drenched Intraperitoneally Subcutaneously	27.10.14, grazing day and night in small Camp C 1.11.14, turned into Camp D 10.11.14, kraaled and given grass and small ration	Negative.
2620	Grazing day and night in Camp D	27.10.14 18.11.14 19.12.14 22.1.15 17.2.15	Coarse lamziekte muscle..... Coarse lamziekte muscle..... 20 c.c. coarse lamziekte muscle..... 2 pints fine lamziekte muscle..... Fine lamziekte muscle.....	Ox 2505 Heifer 2841 Bull 3015 Ox 2262 Cow 2604	Subcutaneously Intraperitoneally Intrajugularly Drenched Intramuscularly	27.10.14, grazing day and night in small Camp C 1.11.14, turned into Camp D 10.11.14, kraaled and given grass and small ration	Negative.

TABLE No. VI.—*Muscle Injection Experiment—(continued).*

No. of Animal.	Previous History.	Date of Inoculation.	Material Used.	Animals from which Material Obtained.	Method of Inoculation.	Treatment received during Period of Experiment.	Result.
2302	Grazing day and night in Camp D	27. 10. 14	Coarse lamziekte muscle.....	Ox 2505	Intraperitoneally	27. 10. 14, grazing day and night in small Camp C	Died per acute Lamziekte (?) 15. 11. 14.
2832	Grazing day and night in Camp D	27. 10. 14 18. 11. 14 19. 12. 14 22. 1. 15 17. 2. 15	2 pints fine lamziekte muscle... Fine lamziekte muscle... 20 c.c. fine lamziekte muscle... Fine lamziekte muscle... Coarse lamziekte muscle...	Ox 2505 Heifer 2841 Bull 3015 Ox 2262 Cow 2604	Drenched Intramuscularly Intraperitoneally Intrajugularly Subcutaneously	27. 10. 14, grazing day and night in small Camp C 1. 11. 14, turned into Camp D 10. 11. 14, kraaled and given grass and small ration	Negative.
2885	Running in veeld.....	27. 10. 14 18. 11. 14 19. 12. 14	2 pints coarse lamziekte muscle. Coarse lamziekte muscle... 20 c.c. coarse lamziekte muscle.	Ox 2505 Heifer 2841 Bull 3015	Drenched Intramuscularly Intraperitoneally	27. 10. 14, grazing day and night in small Camp C 1. 11. 14, turned into Camp D 10. 11. 14, kraaled and given grass and small ration	Died Tympanitis 25. 12. 14.
2891	Control, grazing day and night in Camp D	18. 11. 14 19. 12. 14	2 pints coarse lamziekte muscle. 20 c.c. coarse lamziekte muscle.	Heifer 2841 Bull 3015	Drenched Intramuscularly	17. 11. 14, kraaled and given grass and small ration	Died acute Lamziekte (?) 27. 12. 14.
3244	13. 12. 14, arrived Armoedsvlaakte from Pienaars River	16. 12. 14	10 c.c. coarse and 10 c.c. fine lamziekte muscle	Ox 2274	Intraperitoneally	13. 12. 14, kraaled and fed on hay and small ration 16. 12. 14, transferred to stable	Died Lamziekte acute (?) 24. 12. 14.
3238	13. 12. 14, arrived Armoedsvlaakte from Pienaars River	16. 12. 14 22. 1. 15	10 c.c. coarse and 10 c.c. fine lamziekte muscle	Ox 2274 Ox 2262	Intraperitoneally Intrajugularly	13. 12. 14, kraaled and fed on hay and small ration 16. 12. 14, transferred to stable	Died from shock 22. 1. 15.
3221	13. 12. 14, arrived Armoedsvlaakte from Pienaars River	16. 12. 14 22. 1. 15 17. 2. 15	10 c.c. coarse and 10 c.c. fine lamziekte muscle 10 c.c. coarse and 10 c.c. fine lamziekte muscle Lamziekte muscle.....	Ox 2274 Ox 2262 Cow 2604	Intraperitoneally Subcutaneously Intrajugularly	13. 12. 14, kraaled and fed on hay and small ration 16. 12. 14, transferred to stable	Negative.
3255	13. 12. 14, arrived Armoedsvlaakte from Pienaars River	16. 12. 14 22. 1. 15 17. 2. 15	10 c.c. coarse and 10 c.c. fine lamziekte muscle 10 c.c. coarse and 10 c.c. fine lamziekte muscle Lamziekte muscle.....	Ox 2274 Ox 2262 Cow 2604	Intrajugularly Subcutaneously Intraperitoneally	13. 12. 14, kraaled and fed on hay and small ration 16. 12. 14, transferred to stable	Negative.
3219	13. 12. 14, arrived Armoedsvlaakte from Pienaars River	16. 12. 14 22. 1. 15 17. 2. 15	10 c.c. coarse and 10 c.c. fine lamziekte muscle 10 c.c. coarse and 10 c.c. fine lamziekte muscle Lamziekte muscle.....	Ox 2274 Ox 2262 Cow 2604	Intrajugularly Subcutaneously Intraperitoneally	13. 12. 14, kraaled and fed on hay and small ration 16. 12. 14, transferred to stable	Negative.

TABLE No. VI.—*Muscle Injection Experiment*—(continued).

No. of Animal.	Previous History.	Date of Inoculation.	Material Used.	Animals from which Material Obtained.	Method of Inoculation.	Treatment received during Period of Experiment.	Result.
3251	13.12.14, arrived Armoedsvlakte from Pienaars River	16.12.14 22. 1.15 17. 2.15	10 c.c. coarse and 10 c.c. fine lam-ziekte muscle 10 c.c. coarse and 10 c.c. fine lam-ziekte muscle Lamziekte muscle.....	Ox 2274 Ox 2262 Cow 2604	Intrajugularly Intraparitoneally Subcutaneously	13.12.14, kraaled and fed on hay and small ration 16.12.14, transferred to stable	Negative.
3331	13.12.14, arrived Armoedsvlakte from Pienaars River	16.12.14 22. 1.15 17. 2.15	10 c.c. coarse and 10 c.c. fine lam-ziekte muscle 10 c.c. coarse and 10 c.c. fine lam-ziekte muscle Lamziekte muscle.....	Ox 2274 Ox 2262 Cow 2604	Subcutaneously Intraparitoneally Subcutaneously	13.12.14, kraaled and fed on hay and small ration 16.12.14, transferred to stable	Negative.
3252	13.12.14, arrived Armoedsvlakte from Pienaars River	16.12.14 22. 1.15	10 c.c. coarse and 10 c.c. fine lam-ziekte muscle 20 c.c. coarse and fine lamziekte muscle	Ox 2274 Ox 2262	Subcutaneously Intrajugularly	13.12.14, kraaled and fed on hay and small ration 16.12.14, transferred to stable	Died from shock 22.1.15.
3330	13.12.14, arrived Armoedsvlakte from Pienaars River	16.12.14 22. 1.15 17. 2.15	20 c.c. tongue..... 10 c.c. coarse and 10 c.c. fine lam-ziekte muscle Lamziekte muscle.....	Ox 2274 Ox 2262 Cow 2604	Subcutaneously Intraparitoneally Intrajugularly	13.12.14, kraaled and fed on hay and small ration 16.12.14, transferred to stable	Negative.
2892	Control, grazing day and night in Camp D	19.12.14 22. 1.15 17. 2.15	20 c.c. fine lamziekte muscle... 2 pns coarse lamziekte muscle. Coarse lamziekte muscle.....	Bull 3015 Ox 2262 Cow 2604	Subcutaneously Drenched Intrajugularly	29.11.14, kraaled day and night and fed on grass and small ration	Negative.
2888	Control, grazing day and night in Camp C	19.12.14	20 c.c. fine lamziekte muscle...	Bull 3015	Intrajugularly	29.11.14, kraaled day and night and fed on grass and small ration	Died from shock 19.12.14.
3220	13.12.14, arrived Armoedsvlakte from Pienaars River	19.12.14 22. 1.15	20 c.c. coarse and fine lamziekte muscle 20 c.c. coarse and fine lamziekte muscle	Bull 3015 Ox 2262	Subcutaneously Intrajugularly	13.12.14, kraaled day and night and fed on hay and small ration 16.12.14, transferred to stable	Died from shock 19.12.14.
2420	Control grazing day and night in Camp C	19.12.14	20 c.c. coarse lamziekte muscle.	Bull 3015	Subcutaneously	29.11.14, kraaled day and night and given grass and small ration	Died acute Lamziekte (?) 24.12.14.
3243	13.12.14, arrived Armoedsvlakte from Pienaars River	19.12.14	20 c.c. coarse and fine lamziekte muscle	Bull 3015	Subcutaneously	13.12.14, kraaled and fed on hay and small ration 16.12.14, transferred to stable	Died acute Lamziekte (?) 11.1.15.

TABLE NO. VI.—*Muscle Injection Experiment*—(continued).

No. of Animal.	Previous History.	Date of Inoculation.	Material Used.	Animals from which Material Obtained.	Method of Inoculation.	Treatment received during Period of Experiment.	Result.
2434	20.11.14, grazing day and night in Camp B	22 1.15 17. 2.15	Fine lamziekte muscle..... Fine lamziekte muscle, 2 pints..	Ox 2262 Cow 2604	Intramuscularly Drenched	1.1.15, kraaled and fed on grass and small ration	Negative.
2437	Control, grazing day and night in Camp B	22 1.15 17. 2.15	Fine lamziekte muscle..... Fine lamziekte muscle.....	Ox 2262 Cow 2604	Subcutaneously Intrajugularly	29.12.14, kraaled and fed on grass and small ration	Negative.
3282	Grazing day and night in Camp B	22 1.15 17. 2.15	Fine lamziekte muscle..... Fine lamziekte muscle.....	Ox 2262 Cow 2604	Intrajugularly Intraperitoneally	29.12.14, kraaled and fed on grass and small ration	Negative.
2893	Control, grazing in Camp B day and night	22 1.15 17. 2.15	10 c.c. coarse lamziekte muscle. 2 pints lamziekte muscle.....	Ox 2262 Cow 2604	Intramuscularly Drenched	29.12.14, kraaled and given grass and small ration	Negative.
2637	Blood inoculation experiment running in Camp B	22 1.15 17. 2.15	10 c.c. coarse lamziekte muscle. Coarse lamziekte muscle.....	Ox 2262 Cow 2604	Intraperitoneally Intrajugularly	29.12.14, kraaled and given grass and small ration	Negative.

Regarding these cases, the following further notes may be added: (1) No. 3036 was inoculated intrajugularly on 27th October, 1914, and on 28th October, 1914, a swelling was present at the seat of inoculation. On 30th October, 1914, a swelling was present in the region of the parotid gland, and the animal had a temperature of 103.2. On 10th November, 1914, it was stabled, and on 18th November, 1914, received a further injection of muscle emulsion. On 24th November, 1914, i.e. fourteen days after stabling and six days after the second inoculation, it was found down in the stable. Mr. De Kock remarked: "Later in the day this ox was lying on its right side, showing gasping movements of the jaws, every now and again struggling, but unable to rise. It died about 6 p.m." No definite diagnosis was made. (2) No. 2273 showed swellings and abscesses at the seat of inoculation, and on the 30th a temperature of 103.8. On 24th November, 1914, i.e. fourteen days after stabling and six days after the second inoculation, the animal "was showing similar symptoms of the jaw as described above, with salivation, the food just remaining in the mouth. . . . This ox later in the day was found lying on the sternum, not feeding, and showing quivering of the whole body, temperature 99.8." It died the same night of suspected Lamziekte. (3) No. 2882 showed a swelling in the region of the parotid gland, and on the 30th a temperature of 104. On 24th November, 1914, i.e. fourteen days after stabling and six days after the second inoculation, the animal "appeared to be feeding, but was unable to swallow; there was great effort and good salivation, but the food just remained in the mouth." Again, on 25th November, 1914, Mr. De Kock remarked: "It chews a mouthful of food for a considerable time, with a great amount of white, foamy saliva escaping." The animal died during the night of the 26th. Acute Lamziekte was suspected. (4) No. 2302 was found dead on 15th November, 1914, i.e. five days after it was stabled and eighteen days after inoculation. Peracute Lamziekte was suspected. (5) No. 2891 was found lying down on its side on the afternoon of 26th November, 1914, about five weeks after it was kraaled and seven days after the second inoculation. Mr. De Kock made the following notes: "Slight swelling at seat of inoculation, temperature 97, slight nasal discharge, respirations somewhat laboured." It died on the morning of the 27th, and acute Lamziekte was suspected. (6) No. 3244 arrived on the farm from Pienaars River, a non-lamziekte area, on the 13th December, 1914, and was kraaled on arrival. On 22nd December, 1914, i.e. nine days after its arrival, and six days after inoculation, it showed symptoms which were described as follows:—"This evening the animal refused its food and did not drink, temperature 102.6 in the morning. She stands about listlessly, back somewhat arched, salivation, eyelids drooping, slight lachrymation." It died on the morning of the 24th, and acute Lamziekte was suspected. (7) No. 2420 was noticed ill on 24th December, 1914, i.e. twenty-five days after kraaling and five days after the inoculation. It was found lying on its side in the morning, and later in the day was "tympanitic and exhausted." It died at 2.30 p.m., and peracute Lamziekte was suspected. (8) No. 3243, a Pienaars River animal, was noticed ill on 9th January, 1915, i.e. twenty-seven days after its arrival on the farm, and twenty-one days after the inoculation. The following notes were made by Mr. De Kock:—"This animal since its arrival has been of a lazy and dull disposition. Its feeding and drinking have always been irregular. . . . This evening it was reported not to have

taken any water. Its feeding is done in a very listless way; there is some salivation. During the day it was inclined to lie down frequently. Diagnosis difficult to make at present." The animal died on 11th January, 1915, and Lamziekte was suspected.

Discussion of Results.

The results from these experiments will be discussed more fully later on, but it is necessary here to refer to some points. Considering the results purely from the standpoint of a specific infection, one cannot understand the failure to transmit the infection in such a large number of cases. Accepting that the organisms have invariably been present in the muscles, only 27 per cent. of the animals which were inoculated died subsequently, and this in spite of the fact that some cattle received no fewer than five inoculations at different times with material from different cases of Lamziekte. A specific infectious disease has a fairly definite period of incubation, but in these transmission experiments we find an incubation period varying from five to twenty-one days. Considering the results from the point of view of Lamziekte, one might admit that the rapid course of the disease somewhat resembled that of Lamziekte, but there are good reasons for regarding it not to be identical with Lamziekte, especially as in the experiments to be described next not a single case of the disease could be produced by the same methods of inoculation. The symptoms described are those of a toxæmia, and do not correspond exactly with the symptoms of Lamziekte; the illness appeared to be more acute, and the profuse salivation with inability to swallow, which was such a prominent symptom in these cases, is met with only in the minority of cases of Lamziekte. Assuming the diagnosis of Lamziekte to have been correct, then there would be only two cases to consider seriously, namely, those animals which arrived from Pienaars River, a non-lamziekte area. The other six fatal cases were running on Lamziekte veld, all prior to and some during the transmission experiments, and, as would be seen elsewhere in this paper, we have reason to believe that Lamziekte has a fairly long "incubation" or latent period. If these six animals did die from Lamziekte, therefore, it would not be correct to say definitely that they contracted the disease as a result of the inoculation. More recent observations have shown that animals brought from a non-lamziekte to a lamziekte farm do not appear to be very susceptible to the disease, and, in fact, the shortest period in which such animals showed symptoms of the disease was three and three-quarter months after arrival on the lamziekte farm. In the case of the two fatal cases from Pienaars River, it will be seen that one animal showed symptoms only nine days and the other twenty-seven days after arrival on the lamziekte farm, so that from the point of view of Lamziekte these two cases are difficult to explain or even to understand.

Experiment No. 34—Inoculation with Organs.—These experiments were conducted at Onderstepoort Laboratory under the personal supervision of Sir Arnold Theiler, Director of Veterinary Research. The main object was to control the results of similar experiments carried out at Armoedsvlakte Experimental Station, where a certain amount of danger from natural "infection" was always present. The methods of inoculation employed at Onderstepoort were similar to those already described in the previous experiments, and the materials were obtained from the same source, namely, Armoedsvlakte

Experimental Station. In a few cases the animals were inoculated with material which was preserved in 80 per cent. glycerine, the glycerinated muscle and liver emulsions being prepared at Armoedsvlakte from where they were dispatched to Onderstepoort by rail. The majority of animals, however, received emulsion of muscle which was prepared fresh at Onderstepoort, the muscle being taken from animals sent by rail from Armoedsvlakte. As even acute cases of the disease very often live for two or three days or longer, it was possible to get the sick animal to Onderstepoort alive, provided it was dispatched by rail as soon as a definite diagnosis of Lamziekte was made. Details of these transmission experiments are shown in Table No. VII.

TABLE NO. VII.

Inoculations with Emulsions of Liver and Muscles.

No. of Animal.	Date of Inoculation.	Material Used.	Animals from which Material obtained.	Method of Inoculation.	Result.
3256	24.6.14	75 c.c. alcoholic extract of lamziekte liver	—	Intramuscularly	Negative.
3328	22.6.14	750 c.c. lamziekte liver emulsion	—	Intramuscularly	Malignant oedema; died 26.6.14.
3375	12.1.15	20 c.c. coarse lamziekte muscle	Ox 9	Subcutaneously	Died of shock 12.1.15.
	"	" " "	Ox 9	Intrajugularly	
3377	12.1.15	20 c.c. coarse lamziekte muscle	Ox 9	Subcutaneously	Died of shock 12.1.15.
	"	" " "	Ox 9	Intrajugularly	
3399	12.1.15	20 c.c. coarse lamziekte muscle	Ox 9	Subcutaneously	Died of shock 12.1.15.
	"	" " "	Ox 9	Intrajugularly	
3376	12.1.15	10 c.c. fine lamziekte muscle..	Ox 9	Intrajugularly	Negative.
	4.2.15	20 c.c. fine lamziekte muscle..	Ox 9	Subcutaneously	
	6.3.15	10 c.c. fine lamziekte muscle..	Ox 2432	Intrajugularly	
	"	30 c.c. fine lamziekte muscle..	Ox 2432	Subcutaneously	
3379	12.1.15	10 c.c. fine lamziekte muscle..	Ox 9	Intrajugularly	Negative.
	4.2.15	20 c.c. fine lamziekte muscle..	Ox 9	Subcutaneously	
	6.3.15	10 c.c. fine lamziekte muscle..	Ox 2432	Intrajugularly	
	"	50 c.c. fine lamziekte muscle..	Ox 2432	Subcutaneously	
3380	12.1.15	10 c.c. fine lamziekte muscle..	Ox 9	Intrajugularly	Negative.
	4.2.15	20 c.c. fine lamziekte muscle..	Ox 9	Subcutaneously	
	6.3.15	10 c.c. fine lamziekte muscle..	Ox 2432	Intrajugularly	
5396	12.1.15	10 c.c. fine lamziekte muscle..	Ox 9	Intrajugularly	Negative.
	4.2.15	1 lb. fine lamziekte muscle..	Ox 9	Subcutaneously	
	6.3.15	10 c.c. fine lamziekte muscle..	Ox 2432	Drenched	
	"	30 c.c. fine lamziekte muscle..	Ox 2432	Intrajugularly	
	"	"	Ox 2432	Subcutaneously	
3406	12.1.15	10 c.c. fine lamziekte muscle..	Ox 9	Intrajugularly	Negative.
	4.2.15	20 c.c. fine lamziekte muscle..	Ox 9	Subcutaneously	
	6.3.15	10 c.c. fine lamziekte muscle..	Ox 2432	Intrajugularly	
	"	30 c.c. fine lamziekte muscle..	Ox 2432	Subcutaneously	
3402	12.1.15	10 c.c. fine lamziekte muscle..	Ox 9	Intrajugularly	Negative.
	4.2.15	1 lb. fine lamziekte muscle..	Ox 9	Subcutaneously	
	6.3.15	10 c.c. fine lamziekte muscle..	Ox 2432	Drenched	
	"	50 c.c. fine lamziekte muscle..	Ox 2432	Intrajugularly	
	"	"	Ox 2432	Subcutaneously	
3408	12.1.15	10 c.c. fine lamziekte muscle..	Ox 9	Intrajugularly	Negative.
	4.2.15	50 c.c. fine lamziekte muscle..	Ox 9	Subcutaneously	
	6.3.15	10 c.c. fine lamziekte muscle..	Ox 2432	Intrajugularly	

TABLE NO. VII—(continued).
Inoculations with Emulsions of Liver and Muscles—(continued).

No. of Animal.	Date of Inoculation.	Material Used.	Animals from which Material obtained.	Method of Inoculation.	Result.
2683	25.1.15 " " 4.2.15 6.3.15 " "	10 c.c. fine lamziekte muscle in 80 per cent. glycerine 30 c.c. fine lamziekte muscle in 80 per cent. glycerine 20 c.c. fine lamziekte muscle.. 10 c.c. fine lamziekte muscle.. 50 c.c. fine lamziekte muscle..	Cattle, Vryburg Cattle, Vryburg Ox 2432 Ox 2432	Intrajugularly Subcutaneously Intrajugularly " " Subcutaneously	Negative.
3327	25.1.15 " " 4.2.15 6.3.15 " "	10 c.c. fine lamziekte muscle in 80 per cent. glycerine 30 c.c. fine lamziekte muscle in 80 per cent. glycerine 20 c.c. fine lamziekte muscle.. 10 c.c. fine lamziekte muscle.. 30 c.c. fine lamziekte muscle..	Cattle, Vryburg Cattle, Vryburg Ox 2432 Ox 2432	Intrajugularly Subcutaneously Intrajugularly " " " "	Negative.
3398	25.1.15 4.2.15 6.3.15 " "	100 c.c. fine lamziekte muscle in 80 per cent. glycerine 10 c.c. fine lamziekte muscle.. 50 "c. fine lamziekte muscle..	Cattle, Vryburg 2629 Ox 2432 Ox 2432	Subcutaneously Intrajugularly Subcutaneously	Negative.
3394	25.1.15	100 c.c. fine lamziekte muscle in 80 per cent. glycerine	Cattle, Vryburg	Subcutaneously	Died of Malignant Oedema.
3250	4.2.15 6.3.15 " "	20 c.c. fine lamziekte muscle.. 10 c.c. fine lamziekte muscle.. 30 c.c. fine lamziekte muscle..	2629 Ox 2432 Ox 2432	Intrajugularly " " Subcutaneously	Negative.
3403	4.2.15 6.3.15 " "	20 c.c. fine lamziekte muscle.. 10 c.c. fine lamziekte muscle.. 30 c.c. fine lamziekte muscle..	2629 Ox 2432 Ox 2432	Intrajugularly " " Subcutaneously	Negative.
3404	4.3.15 6.3.15 " "	20 c.c. fine lamziekte muscle.. 10 c.c. fine lamziekte muscle.. 50 c.c. fine lamziekte muscle..	2629 Ox 2432 Ox 2432	Intrajugularly " " Subcutaneously	Negative.
3400	4.2.15 6.3.15 " "	100 c.c. fine lamziekte muscle. 10 c.c. fine lamziekte muscle. 30 c.c. fine lamziekte muscle.	2629 Ox 2432 Ox 2432	Subcutaneously Intrajugularly Subcutaneously	Negative
3412	4.2.15 6.3.15 " "	100 c.c. fine lamziekte muscle. 10 c.c. fine lamziekte muscle. 50 c.c. fine lamziekte muscle.	2629 Ox 2432 Ox 2432	Subcutaneously Intrajugularly Subcutaneously	Negative.
3413	4.2.15 6.3.15	100 c.c. fine lamziekte muscle. 10 c.c. fine lamziekte muscle.	2629 Ox 2432	Subcutaneously Intrajugularly	Negative.
2569	4.2.15 6.3.15 " "	$\frac{1}{2}$ lb. fine lamziekte muscle.... 10 c.c. fine lamziekte muscle.. 50 c.c. fine lamziekte muscle..	2629 Ox 2432 Ox 2432	Drenched Intrajugularly Subcutaneously	Negative.
2577	4.2.15 6.3.15 " "	$\frac{1}{2}$ lb. fine lamziekte muscle.... 10 c.c. fine lamziekte muscle.. 50 c.c. fine lamziekte muscle..	2629 Ox 2432 Ox 2432	Drenched Intrajugularly Subcutaneously	Negative.

Discussion of Results.

As far as Lamziekte is concerned, the results were entirely negative, no symptoms of any disease resembling Lamziekte in any way being produced in the inoculated animals. As was the case in the Armoedsvlakte experiments, some animals died from shock, and two contracted malignant oedema. The experiments at Onderstepoort were conducted on nearly as large a scale as those on Armoedsvlakte, twenty-four animals being inoculated with material from five cases of Lamziekte, as compared with thirty animals inoculated with material from six cases on Armoedsvlakte. The experiments at Onderstepoort were carried out with animals which had never been on a lamziekte

farm, and under the best conditions as regards stabling accommodation, laboratory equipment, facilities for sterilizing materials, etc., whereas on Armoedsvlakte no such facilities were present, and most of the animals had been running on lamziekte farms prior to the commencement of the experiments. When these facts are taken into consideration, it is perhaps possible to explain some of the Armoedsvlakte cases, at any rate, as a toxæmia resulting from accidental infection, and others as cases of Lamziekte contracted naturally on lamziekte veld prior to the inoculations. As already pointed out, there were no facilities on Armoedsvlakte for carrying out proper bacterial examinations. If the disease could be transmitted by the inoculation of muscle from cases of Lamziekte, surely one could have expected some positive results from the Onderstepoort experiments! Moreover, one would be at a complete loss to explain the natural method of infection, as also the peculiarly intimate connection between the incidence of the disease and the climatic and veld conditions. From the Sarcosporidia point of view it will be seen in another paper that Lamziekte has been observed in a young animal whose muscles did not harbour a single parasite, and that the parasites usually occur in much smaller numbers in animals dead from Lamziekte than in the cases of poverty and senility. It is unnecessary to discuss these results any further, as the transmission experiments are to be repeated on Armoedsvlakte, where proper laboratory accommodation is now being erected and stabling accommodation has already been provided.

Experiment No. 35.—Drenching with Fly Pupae.—The object of this experiment was to find out whether Lamziekte could be produced by drenching animals with fly pupae (*Pycnosoma marginale*) which had been reared on carcasses of animals which had died from Lamziekte. For the purpose of collecting the fly pupae, lamziekte carcasses were deposited in one corner of paddock "C," the development of the larvae was watched, and as soon as they had changed into pupae the collection was made and the pupae were forwarded to Onderstepoort. Most of the drenching experiments were, therefore, carried out at Onderstepoort under the personal supervision of Sir Arnold Theiler, only one animal (No. 3327) being under treatment on Armoedsvlakte.

Details of the experiments are shown in Table No. VIII.

TABLE NO. VIII.
Drenching with Fly Pupae.

No. of Animal.	Date of Drenching.	Material Used.	Source from which Material obtained.	Result.
3519	13. 3. 15	800 pupae of <i>Pycnosoma marginale</i>	Reared on lamziekte carcasses, Armoedsvlakte	Died, Enteritis (Lamziekte ?), 25.3.15.
2569	7. 4. 15	800 pupae of <i>Pycnosoma</i> sp.	Reared on carcass of 3519....	Negative.
3327	7. 4. 15	800 pupae of <i>Pycnosoma</i> sp.	Reared on carcass of 3519....	Negative.
2577	7. 4. 15	800 pupae of <i>Pycnosoma</i> sp.	Reared on carcass of 3519....	Negative.
3470	7. 4. 15	800 pupae of <i>Pycnosoma</i> sp.	Reared on carcass of 3519....	Negative.
3402	7. 4. 15	800 pupae of <i>Pycnosoma marginale</i>	Reared on carcass of 3519....	Negative.
3407	7. 4. 15	800 pupae of <i>Pycnosoma marginale</i>	Reared on carcass of 3519....	Negative.

TABLE No. VIII—(continued).
Drenching with Fly Pupae—(continued).

No. of Animal.	Date of Drenching.	Material used.	Source from which Material obtained.	Result.
3413	7. 4.15	800 pupae of <i>Pycnosoma marginale</i>	Reared on carcass of 3519...	Negative.
3516	18. 5.15	600 pupae of <i>Pycnosoma</i> sp.	Reared on lamziekte carcass, Armoedsvlakte	Negative.
	22. 5.15	900 pupae of <i>Pycnosoma</i> sp.	" " "	
	17. 9.15	" " "	" " "	
	9.10.15	" " "	" " "	
	12.10.15	" " "	" " "	
3527	18. 5.15	600 pupae of <i>Pycnosoma</i> sp.	Reared on lamziekte carcass, Armoedsvlakte	Negative.
	22. 5.15	900 pupae of <i>Pycnosoma</i> sp.	" " "	
3523	18. 5.15	600 pupae of <i>Pycnosoma</i> sp.	Reared on lamziekte carcass, Armoedsvlakte	Died Toxaemia. 29.5.15.
	22. 5.15	900 pupae of <i>Pycnosoma</i> sp.	" " "	
3525	18. 5.15	600 pupae of <i>Pycnosoma</i> sp.	Reared on lamziekte carcass, Armoedsvlakte	Negative.
	22. 5.15	900 pupae of <i>Pycnosoma</i> sp.	" " "	
3327	7.12.15	3 oz. pupae.....	Reared on lamziekte carcass, Armoedsvlakte	Negative.

Discussion of Results.

A total number of thirteen animals was drenched with fly pupae, and two of these died from Toxaemia, resulting from the ingestion of pupae. Cow No. 3519 was drenched on 13th March, 1915, and died on 26th March, 1915, when the following notes were made by Sir Arnold Theiler: "Native states that since last evening the animal has not been well. This morning it was down, lying in the sternal position with its head inclined over to its side; cannot stand up when forced to, and when lifted is unable to keep on its legs; soon after it was stretched out and passed plenty of urine. Temperature was normal up to death, and shortly before it was 101° F. It looked fairly bright, and slight salivation was present. Death must have occurred shortly after."

The carcass of this animal was used as a rearing ground for fly pupae, and seven other cattle were drenched with pupae so obtained, with negative results in all cases.

No. 3523 was drenched with pupae on 18th May, 1915, and again on 22nd May, 1915, and on 25th May, 1915, was noticed to be ill. The following notes were made by Sir Arnold Theiler: "On 25th noticed not to be quite well, leaving its food, and saliva hanging about the mouth. On 26th is able to stand, it tries to feed, but the apprehension of food seems to be difficult; animal moves its head in the food, stretches out the tongue slightly, moves it to and fro, but seems unable to take in a larger quantity of food. When explored by the hand a cud is found sticking far back, which the animal apparently tried to swallow but could not. The food was now changed to bran mash. On 27th the animal tries to eat the mash, but cannot get it into the mouth, it protrudes the tongue but slightly, but cannot grip the food, neither does it seem capable of sucking it in. The stable wall in front of the animal is besmeared with bran, the animal stretching out the head, moving its tongue slightly, and so throwing the

food about its head. When exploring, nothing abnormal is found in the mouth. There is still some liquid present in the mouth, which collects at the root of the tongue, escapes into the pharynx, and causes the animal to cough. . . . The animal is losing condition rapidly and is weak. 26th: Condition much the same, tongue slightly protruding. Animal is able to rise, but lies most of the time, dull, apathetic, drooping ears, half-closed eyes, fallen in at flanks. It died on 29th." It is interesting to note that three other animals (Nos. 3516, 3527, 3525) received the same quantities of pupae on the same dates as No. 3523, but with negative results in all cases. In both these cases again paralysis of the tongue and throat muscles, with profuse salivation, was one of the most prominent symptoms, and, as mentioned in the discussion on the results from Experiment No. 33, this symptom appears only in the small minority of cases of Lamziekte. Considering the very putrid material on which these pupae were reared, it would not be at all surprising if the ingestion of large quantities of them caused toxæmia in cattle. On farms where all carcasses are carefully disposed of, it would be an extremely difficult matter to find any fly pupae in the veld, and yet Lamziekte is contracted on such veld. Moreover, the disease has been contracted by animals running in small paddocks on Armoedsvlakte, where no carcasses nor any fly pupae were ever observed. Apart from Lamziekte, the results of these experiments were very interesting, so much so that fly pupae will receive further consideration.

Experiment No. 36—Drenching with Insects.—The object of these experiments, which were carried out both on Armoedsvlakte and at Onderstepoort, was to find out whether Lamziekte could be produced by drenching cattle with insects collected on a lamziekte farm, remains of such insects having often been discovered in the stomachs of cattle which have died from Lamziekte. The drenching with insects could not be carried out on a very extensive scale, for the simple reason that the collection of such insects in sufficiently large quantities is not an easy matter.

Table No. IX shows the details of the experiments, the results of which were entirely negative, and require no further discussion.

TABLE NO. IX.

Drenching with Insects.

No. of Animal.	Date of Drenching.	Material Used.	Where Material Obtained.	Result.
3411	3. 5.15	Ground-up beetles.....	Armoedsvlakte, Vryburg....	Negative.
3524	3. 5.15	Ground-up beetles.....	Armoedsvlakte, Vryburg....	Negative.
3397	9. 1.16 7. 2.16	Ground-up beetles..... " "	Armoedsvlakte, Vryburg.... " " "	Negative.
3383	7. 2.16	Ground-up beetles.....	Armoedsvlakte, Vryburg....	Negative.
3524	14.10.15	Drench with red ants.....	Armoedsvlakte, Vryburg	Negative.
3092	24. 2.16 2. 3.16 3. 3.16	Drenched with black ants.... " " " "	Armoedsvlakte, Vryburg.... " " "	Negative.
2577	4. 7.16 14. 7.16	Drenched with ants..... " "	Armoedsvlakte, Vryburg.... " " "	Negative.

Experiment No. 37—Feeding of Faeces.—The object of this experiment was to find out whether Lamziekte could be produced by feeding animals on faeces voided by animals suffering from an acute attack of the disease. The results of previous experiments in which healthy animals were drenched with the stomach contents of lamziekte animals were entirely negative. After collection the faeces were allowed to dry, and then crushed, powdered, and mixed with bran and maize for feeding. Cow No. 2939 was fed daily on faeces for ten days, from 14th July, 1916, to 23rd July, 1916, but the ration was not taken very readily. Cow No. 1148 received a daily ration of faeces for eighteen days, from 24th July, 1916, to 10th August, 1916, and excepting for the first day the ration was consumed very readily. The result in both cases was entirely negative.

Remarks.—The experiment was not carried out on a sufficiently large scale to allow of definite conclusions to be drawn from the results, and will be repeated at a later date.

Experiment No. 38—Blood Inoculation.—A few years ago considerable importance was attached to a method of preventive inoculation against Lamziekte advocated by Dr. Matthias, of Schweizer Reneke. Briefly described, his method was as follows:—An animal suffering from Lamziekte, preferably one which had been down for several days, was bled from the jugular vein into a clean vessel, the blood during withdrawal being stirred constantly with a wisp to prevent clotting. The defibrinated blood so obtained was then used for inoculating healthy cattle, 20 c.c. being injected subcutaneously. It was alleged that animals inoculated in this way would not contract Lamziekte for a period of about six months. As regards the nature of the immunity alleged to be conferred on animals by this inoculation, the following is an extract from a report by Mr. W. H. Andrews, Veterinary Research Officer, who had a personal interview with Dr. Matthias:—"Dr. Matthias considers that the inoculation actually causes the transmission of the disease, which appears in a mild form under these conditions, and he explains the partial failures by assuming that the material was taken at an unfavourable stage of the disease. He compares the disease with the pneumonia of mine compounds, in which disease recovery from a natural attack is accompanied by the development of an immunity lasting only for about six months, the patient showing later on increased susceptibility to the disease."

Although from a scientific point of view and from the point of view of our knowledge of the disease, we could not agree with the theory advanced by Dr. Matthias, it was decided to give his method of inoculation a thorough trial. A total number of 116 head of cattle was inoculated with blood from lamziekte animals on different dates, whilst 119 cattle served as controls. In Tables Nos. X, XA, and XB will be seen particulars of the cases of Lamziekte which were contracted within a year from the time of the inoculation, this period being found the most convenient for the purpose of comparing the results.

TABLE NO. X.

Blood Inoculation Experiment—Showing particulars of cases of Lamziekte amongst 25 Inoculated and 25 Control Animals.

No. of Animal.	Date of Inoculation.	Date on which Symptoms first seen.	Recovery or Death.
2419	14.8.14	14.1.15	Died 18.1.15.
2605	"	8.2.15	Died 10.2.15.
2385	"	2.3.15	Died 4.3.15.
3145	"	9.3.15	Died 10.3.15.
3001	"	19.3.15	Recovered 8.4.15.
3141	"	2.4.15	Died 6.4.15.
2133	"	2.4.15	Recovered 12.4.15.
2648	Control	13.8.14	Recovered 19.8.14.
2604	"	14.2.15	Pithed L. Z. 17.2.15.
2602	"	1.3.15	Recovered 9.3.15.
2432	"	2.3.15	Sent to Pretoria 3.3.15.
3159	"	12.3.15	Recovered 15.3.15.
3042	"	29.4.15	Sent to Pretoria 29.4.15.
2305	"	11.5.15	Pithed 30.6.15; poverty after slow recovery from Lamziekte.
3157	"	1.6.15	Recovered 7.6.15.
2145	"	22.3.15	Died 31.3.15.

TABLE NO. XA.

Blood Inoculation Experiment—Showing particulars of Lamziekte cases amongst 25 Inoculated and 25 Control Animals.

No. of Animal.	Date of Inoculation.	Date on which Symptoms first seen.	Recovery or Death.
2632	19.8.14	18. 9.14	Recovered 25.9.14.
3155	"	2.11.14	Died 3.11.14.
2616	"	25.11.14	Died 27.11.14.
2632	"	15. 2.15	Died 4.3.15.
2973	"	22. 2.15	Died 23.2.15.
2619	"	3. 6.15	Recovered 12.6.15.
2397	Control	23. 5.15	Died 16.6.15.
2841	"	15.11.14	Pithed Lamziekte 18.11.14.
2439	"	5. 1.15	Recovered 18.1.15.
2188	"	Died suddenly	Died 14.3.15.

TABLE No. XB.

Blood Inoculation Experiment—Showing particulars of fatal cases of Lamziekte amongst 66 Inoculated and 69 Control Animals.

No. of Animal.	Date of Inoculation.	Date on which Symptoms first seen.	Date of Death.
2262	27.10.14	20. 1.15	22. 1.15
2264	"	28. 4.15	19. 5.15
2266	"	Died suddenly	17. 9.15
2275	"	22. 3.15	26. 3.15
2445	"	Died suddenly	9. 9.15
2449	"	"	14.11.14
2455	"	"	14. 8.15
2629	"	1. 2.15	2. 2.15
3031	"	21.12.14	25.12.14
3036	"	2. 1.15	5. 1.15
2322	"	25. 2.15	26. 2.15
3132	"	Died suddenly	25.11.14
2268	Controls	17. 4.15	26. 4.15
2274	"	15.12.14	16.12.14
2420	"	24.12.14	24.12.14
2421	"	28.12.14	30.12.14
2426	"	2. 2.15	5. 2.15
2434	"	23. 9.15	26. 9.15
3028	"	24.12.14	26.12.14
2411	"	26.11.14	27.11.14
2450	"	26. 3.15	29. 3.15
2908	"	3. 1.15	7. 1.15

Discussion.

In adding up the number of cases which occurred amongst the inoculated animals and amongst the controls, we find twenty-five cases amongst the inoculated animals and twenty-three amongst the controls, i.e. 22 per cent. in the former and 20 per cent. in the latter, so that actually more cases of the disease occurred amongst the animals which it was sought to immunize. The difference was not sufficiently great to justify any conclusions other than that the method of inoculation failed completely to protect animals against Lamziekte. Reporting on the results of his inquiries into this method of inoculation, Mr. Andrews states further: "It is also held by Dr. Matthias and Mr. McLetchie that the above-mentioned symptoms of general disturbance are manifestations of mild attacks of Gal-lamziekte, directly transmitted by inoculation." The symptoms of general disturbance referred to were dullness in some cases, a lessened yield of milk in others, a staring coat, and local swellings in still other cases. Neither a local swelling nor any systemic disturbance was observed in our experimental animals, which were under daily observation, and of which daily temperature records were kept. The reactions noticed by farmers must, therefore, have been due to factors other than pure lamziekte blood; amongst such factors may be included accidental bacterial contamination, or protozoan infection of the donor's blood. In this connection it may be mentioned that the reaction set up by the inoculation of cattle with redwater and anthrax vaccines has been held to be temporarily effective in warding off attacks of Lamziekte. The figures given in Tables Nos. X, Xa, and Xb are for a period of one year after the inoculation of the different lots of animals, but, as Dr. Matthias is said to claim an immunity resulting from his method

of inoculation lasting only up to six months, we may consider here only cases of Lamziekte which occurred within six months after the inoculation. We then find 12 per cent. of the inoculated animals to have contracted Lamziekte, compared to only 11 per cent. of the controls. The difference in the percentage cases again is a very small one, so that it may be safely concluded that no immunity whatever is conferred on animals by the inoculation of blood taken from an animal whilst suffering from an acute attack of Lamziekte.

Experiment No. 39—Inoculation with Anthrax Vaccine.—In the discussion on the results of the previous experiment, it was mentioned that the inoculation of anthrax vaccine has also been held to ward off attacks of Lamziekte for several months. The information to be given here was not obtained as the result of actual experiments carried out to test the effect of anthrax vaccine on the incidence of Lamziekte, but purely as the result of tests carried out with the vaccine itself. A total number of 283 head of cattle has been inoculated with anthrax vaccine on this station, and subsequent to the inoculation eighteen cases of Lamziekte have occurred. In Table No. XI are shown further particulars of the cases of Lamziekte.

TABLE NO. XI.
Inoculation with Anthrax Vaccine.

No. of Animal.	Date of Inoculation.	Amount Inoculated.	Deaths from Lamziekte.
2313	29. 8.14	1 c.c. anthrax vaccine	Died 25.3.16.
2170	8. 5.16	1 c.c. anthrax vaccine	Died 5.12.16.
2364	13. 1.17	$\frac{1}{2}$ c.c. anthrax vaccine	Pithed 23.3.18.
2428	13. 1.17	$\frac{1}{2}$ c.c. anthrax vaccine	Died 20.4.18.
2647	13. 1.17	$\frac{1}{2}$ c.c. anthrax vaccine	Died 6.5.18.
3098	8. 5.16 13. 1.17	1 c.c. anthrax vaccine $\frac{1}{2}$ c.c. anthrax vaccine	Died 15.12.17.
3251	8. 5.16 22.12.16	1 c.c. anthrax vaccine $\frac{1}{2}$ c.c. anthrax vaccine	Died 18.10.17.
3387	8. 5.16	1 c.c. anthrax vaccine	Died 21.8.16.
3493	8. 5.16	1 c.c. anthrax vaccine	Died 13.8.16.
3602	8. 5.16	1 c.c. anthrax vaccine	Died 11.9.16.
3714	13. 1.17	$\frac{1}{4}$ c.c. anthrax vaccine	Died 17.3.17.
3120	13. 1.17	$\frac{1}{2}$ c.c. anthrax vaccine	Died 30.1.17.
3816	22.12.16	$\frac{1}{2}$ c.c. anthrax vaccine	Died 26.1.17.
3878	13. 1.17	$\frac{1}{2}$ c.c. anthrax vaccine	Died 16.10.17.
3879	13. 1.17	$\frac{1}{2}$ c.c. anthrax vaccine	Died 19.10.17.
2752	13. 1.17	$\frac{1}{2}$ c.c. anthrax vaccine	Died 17.10.17.
3222	13. 1.17	$\frac{1}{2}$ c.c. anthrax vaccine	Died 14.12.17.
3649	13. 1.17	$\frac{1}{2}$ c.c. anthrax vaccine	Died 12.2.18.

Discussion of Results.

Out of a total of 283 animals, eighteen contracted Lamziekte after different periods subsequent to the vaccination, six within six months, seven within twelve months, and five after more than a year after the inoculation. The large majority of animals were inoculated on 13th January, 1917, since when Lamziekte has not been very prevalent on the farm, and this accounted for the rather low percentage (7) of cases. Unfortunately, we have no control animals for the purpose of comparison, but the mere fact that six out of the eighteen animals died within a few months after the inoculation goes to prove that inoculation with anthrax vaccine has no preventive action against an attack of Lamziekte.

Experiment No. 40—Curative Treatment.—The methods of curative treatment to be reported on here were carried out purely as an experiment during the last few years, and in many cases they were the results of favourable reports received from farmers and others who vouched for their efficacy in curing cases of Lamziekte. Strictly speaking, no rational therapeutics could be applied to cases of Lamziekte, for the simple reason that the true cause of the disease was, and still is, unknown to us, but many drugs were administered on the assumption that the disease was caused theoretically by certain agencies; for example, orypan, levurine, and yeast were tested on the assumption that the disease was in the nature of an avitaminosis; salvarsan, arsenophenyl glycine, trypan blue, etc., on the assumption that Lamziekte was caused by bacterial or protozoan infection; pilocarpine, eserine, and arecoline were employed to counteract the constipation and impaction which are sometimes present in cases of Lamziekte; strychnine was used to stimulate the nervous system, whilst chloral hydrate had the opposite effect. In many cases the treatment adopted was undoubtedly empirical. There is no need to add anything further, as all the particulars of the different methods of treatment adopted are shown in Table No. XII.

TABLE NO. XII.
Curative Treatment for Lamziekte.

Nature of Treatment.	Quantity and Method of Administration.	Nos. of Animals Treated.	Date of Treatment.	Result of Treatment.
Arecoline and pilocarpine	1½ grains and 2½ grains injected subcutaneously	2170	2.12.16	Died 5.12.16.
Arecoline and pilocarpine	1½ grains and 2½ grains injected subcutaneously	2579	2.12.16	Died 3.12.16.
Arsenophenyl glycine	11 grm. in 1 lit. water injected intrajugularly	2385	3. 3.15	Died 4.3.15.
Arsenophenyl glycine	11 grm. in 1 lit. water injected intrajugularly	3145	9. 3.15	Died 10.3.15.
Arsenophenyl glycine	9 grm. in 1 lit. water injected intrajugularly	3159	12. 3.15	Recovered.
Arsenophenyl glycine	18 grm. in 1 lit. water injected intrajugularly	2427	15. 3.15	Recovered.
Arsenophenyl glycine	10 grm. in 1 lit. water injected intrajugularly	2397	28. 5.15	Died 16.6.15.
Blood (defibrinated).	850 c.c. injected intrajugularly	2339	17. 5.14	Died 18.5.14.
Blood (defibrinated).	1500 c.c. injected intrajugularly	2623	29. 5.14	Killed 30.5.14.

TABLE NO. XII—(continued).
Curative Treatment for Lamziekte—(continued).

Nature of Treatment.	Quantity and Method of Administration.	Nos. of Animals Treated.	Date of Treatment.	Result of Treatment.
Blood (defibrinated).	1500 c.c. injected intrajugularly	2840	31. 5.14	Died 1.6.14.
Blood (extraction of)	1½ lit. bled from jugular vein...	2328	23. 5.14	Died 24.5.14.
Blood (extraction of)	3 lit. bled from jugular vein..	3878	15.10.17	Died 16.10.17.
Blood (extraction of)	3 lit. bled from jugular vein..	3251	17.10.17	Died 18.10.17.
Blood (extraction of)	3100 c.c. bled from jugular vein	3879	18.10.17	Died 19.10.17.
Blood (extraction of)	4½ lit. bled from jugular vei ..	Cow (Smollan)	27.11.17	Died 28.11.17.
Blood (extraction of)	3½ lit. bled from jugular vein..	3098	11.12.17	Killed 15.12.17.
Blood (extraction of)	3.15 lit. bled from jugular vein	3222	13.12.17	Died 14.12.17.
Blood (extraction of)	2.8 lit. bled from jugular vein	2911	13.12.17	Died 14.12.17.
Chloral hydrate.....	½ oz. drenched..... 1½ oz. in three doses drenched.	2601 2601	16. 9.15 17. 9.15	Died 19.9.15.
Chloral hydrate.....	1½ oz. in three doses drenched 1 oz. in two doses drenched..	2434 2434	24. 9.15 25. 9.15	Died 26.9.15.
Chloral hydrate.....	½ oz. drenched..... 1 oz. drenched..... 1 oz. drenched.....	2416 2416 2416	25.10.15 25.10.15 25.10.15	Died 26.10.15.
Chloral hydrate.....	3 oz. in three doses drenched.. 3 oz. in three doses drenched.. 3 oz. in three doses drenched.. 2 oz. in two doses drenched...	2459 2459 2459 2459	27.10.15 28.10.15 29.10.15 30.10.15	Died 6.11.15.
Cholesterine.....	Emulsion of 10 grm. in 1000 c.c. water injected intrajugularly	2471	18. 6.14	Died 18.6.14.
Cholesterine.....	Emulsion of 10 grm. in 1000 c.c. water injected intrajugularly	2370	10. 7.14	Died 10.7.14.
Cocaine hydrochlor...	1½ grm. injected subcutaneously	3376	26. 8.16	Recovered 30.8.16.
Cocaine hydrochlor...	1½ grm. injected subcutaneously	2353	24. 8.16	Died 26.8.16.
Cocaine hydrochlor...	1½ grm. injected subcutaneously	651	23. 9.16	Died 23.9.16.
Corrosive sublimate and sodium chloride	(5 grm. and 37.5 grm. in 1000 c.c. water) 10 c.c. injected intrajugularly	2130	21.12.14	Recovered.
Corrosive sublimate and sodium chloride	(5 grm. and 37.5 grm. in 1000 c.c. water) 10 c.c. injected intrajugularly	3031	21.12.14	Died 25.12.14.
Corrosive sublimate and sodium chloride	(5 grm. and 37.5 grm. in 1000 c.c. water) 10 c.c. injected intrajugularly	3028	24.12.14	Died 26.12.14.
Corrosive sublimate and sodium chloride	(5 grm. and 37.5 grm. in 1000 c.c. water) 10 c.c. injected intrajugularly	2421	29.12.14	Died 30.12.14.
Curare.....	½ grm. injected subcutaneously	2427	20.11.16	Pithed 1.12.16.
Eserine.....	2 grains injected subcutaneously	3376	2.10.16	Died 4.10.16.
Formalin.....	5 grm. in 1000 c.c. water injected intrajugularly	3141	4. 4.15	Died 6.4.15.
Formalin.....	5 grm. in 1000 c.c. water injected intrajugularly	2133	6. 4.15	Died 12.4.15.
Formalin.....	2½ grm. in 1000 c.c. water injected intrajugularly	2925	27. 4.15	Died 28.4.15.
Iodine and potassium iodide	4 grm. and 5 grm. in 1000 c.c. water injected intrajugularly	3036	3. 1.15	Died 5.1.15.
Iodine and potassium iodide	2 grm. and 5 grm. in 1000 c.c. water injected intrajugularly	2908	3. 1.15	Died 7.1.15.

TABLE NO. XII—(continued).
Curative Treatment for Lamziekte—(continued).

Nature of Treatment.	Quantity and Method of Administration.	Nos. of Animals Treated.	Date of Treatment.	Result of Treatment.
Iodine and potassium iodide	2 grm. and 5 grm. in 1000 c.c. water injected intrajugularly	2418	2. 2.15	Died 3.2.15.
<i>Leonotus leonurus</i> plant	3 pints decoction drenched....	Cow (Fisher)	5. 3.17	Died 10.3.17.
Levurine.....	400 grm. injected.....	2503	13. 3.14	Died 18.3.14.
Methylene blue.....	200 c.c. of a 1 per cent. solution injected intrajugularly	2419	17. 1.15	Died 18.1.15.
Novo flavin.....	2 grm. in 1000 c.c. water injected intrajugularly	2450	26. 3.15	Died 29.3.15.
Orypan.....	50 grm. in 500 c.c. physiological saline solution injected intrajugularly	2162	28. 1.14	Died 31.1.14.
Orypan.....	50 grm. in 500 c.c. water injected intrajugularly	2145	14. 3.14	Recovered.
Orypan.....	10 $\frac{1}{2}$ grm. in 1 bottle water drenched	3148	13. 5.14	Died 14.5.14.
Orypan.....	50 c.c. liquid injected subcutaneously	2987	14. 5.14	Died 15. 5.14.
Orypan.....	50 c.c. liquid drenched.....	2987	14. 5.14	
Orypan.....	100 grm. in solution of 500 c.c. water injected intrajugularly	2989	17. 5.14	Died 20.5.14.
Orypan.....	400 grm. drenched and injected	3166	26. 5.14	Died 30.5.14.
Orypan.....	150 grm. liquid drenched....	3151	4. 6.14	Died 4.6.14.
Orypan.....	200 grm. in solution of 500 c.c. water injected intrajugularly	3151	4. 6.14	
Orypan.....	100 grm. in solution of 500 c.c. water injected intrajugularly	2965	30. 6.14	Died 3.7.14.
Oxygen.....	1200 c.c. injected intrajugularly at rate of 200 c.c. per minute	2323	11. 6.14	Died 13.6.14.
Oxygen.....	1000 c.c. injected intrajugularly at rate of 200 c.c. per minute	2367	23. 6.14	Died 24.6.14.
Oxygen.....	1000 c.c. injected intrajugularly	3134	29. 6.14	Pithed 2.7.14.
Peptone.....	Emulsion of 15 grm. in 600 c.c. water at blood heat injected intrajugularly	2334	21. 8.14	Recovered 25.8.14.
Peptone.....	Emulsion of 15 grm. in 600 c.c. water at blood heat injected intrajugularly	2326	21. 8.14	Died 23.8.14.
Pilocarpine and eserine	2 grains and 1 grain injected subcutaneously	3376	23. 8.16	Recovered 30.8.16.
Pilocarpine and eserine	2 $\frac{1}{2}$ grains and 2 grains injected subcutaneously	2353	24. 8.16	Died 26.8.16.
Pilocarpine and eserine	2 grains and 1 grain injected subcutaneously	3602	10. 9.16	Died 11.9.16.
Pilocarpine and eserine	2 $\frac{1}{2}$ grains and 2 grains injected subcutaneously	651	22. 9.16	Died 23.9.16.
Pilocarpine and eserine	2 $\frac{1}{2}$ grains and 1 grain injected subcutaneously	3723	22. 9.16	Died 23.9.16.
Potassium permanganate	$\frac{1}{2}$ grm. in 500 c.c. water injected intrajugularly	2615	22. 5.14	Died 23.5.14.
Potassium permanganate	1 grm. in 1000 c.c. water injected intrajugularly	2328	22. 5.14	Died 24.5.14.
Potassium permanganate	1 grm. in 1000 c.c. water injected intrajugularly	2623	23. 5.14	Killed 30.5.14.

TABLE NO. XII—(continued).
Curative Treatment for Lamziekte—(continued).

Nature of Treatment.	Quantity and Method of Administration.	Nos. of Animals Treated.	Date of Treatment.	Result of Treatment.
Potassium permanganate	1 grm. in 1000 c.c. water injected intrajugularly	3166	29. 5.14	Died 30.5.14.
Salvarsan.....	1 lit. solution injected intrajugularly	2883	15.10.14	Died 16.10.14.
Salvarsan.....	12 grm. in 1 lit. of physiological saline solution injected intrajugularly	3155	3.11.14	Died 3.11.14.
Salvarsan.....	7 grm. in 1 lit. water injected intrajugularly	2411	26.11.14	Died 27.11.14.
Sodium sulphate.....	500 grm. in 1000 c.c. water injected intrajugularly	2441	19. 5.14	Died 20.5.14.
Sodium sulphate.....	500 grm. in 1000 c.c. water injected intrajugularly	2993	21. 5.14	Died 21.5.14.
Strychnine sulphate..	90 mg. injected subcutaneously	2277	20. 5.16	Died 20.5.16.
Strychnine sulphate..	50 mg. injected subcutaneously	Heifer	20. 6.16	Recovered 27.6.16.
Strychnine sulphate..	50 mg. injected subcutaneously 50 mg. injected subcutaneously	3606 3606	20. 6.16 28. 6.16	Recovered 1.7.16.
Strychnine sulphate..	50 mg. injected subcutaneously 50 mg. injected subcutaneously 25 mg. injected subcutaneously	3593 3593 3593	10. 7.16 11. 7.16 11. 7.16	Died 12.7.16.
Strychnine sulphate..	50 mg. injected subcutaneously 50 mg. injected subcutaneously	3591 3591	16. 7.16 16. 7.16	Died 16.7.16.
Strychnine sulphate..	50 mg. injected subcutaneously	Heifer	4. 8.16	Died 4.8.16.
Strychnine sulphate..	50 mg. injected subcutaneously	3493	11. 8.16	Died 14.8.16.
Trypan blue.....	200 c.c. of a 2 per cent. solution injected intrajugularly	2426	2. 2.15	Died 5.2.15.
Trypan blue.....	125 c.c. of a 2 per cent. solution injected intrajugularly	2334	8. 2.15	Recovered 20.2.15.
Trypan blue.....	150 c.c. of a 2 per cent. solution injected intrajugularly	2459	8. 2.15	Recovered 15.2.15.
Trypan blue.....	200 c.c. of a 1 per cent. solution injected intrajugularly	2605	8. 2.15	Died 10.2.15.
Trypan blue.....	150 c.c. of a 1 per cent. solution injected intrajugularly	2322	25. 2.15	Died 26.2.15.
Trypan blue.....	150 c.c. of a 1 per cent. solution injected intrajugularly	2912	16.11.15	Died 19.11.15.
Trypan blue.....	100 c.c. of a 1 per cent. solution injected intrajugularly	2627	30.11.15	Died 1.2.15.
Trypan blue.....	100 c.c. of a 1 per cent. solution injected intrajugularly	3001	16.12.15	Died 17.12.15.
Trypan blue.....	150 c.c. of a 1 per cent. solution injected intrajugularly	2444	6. 1.16	Died 8.1.16.
Trypan blue.....	150 c.c. of a 1 per cent. solution injected intrajugularly	3502	20.5.16	Recovered 25.5.16.
Yeast.....	8 oz. drenched..... 4 oz. in 1 bottle water drenched	2965 2965	1. 7.14 2. 7.14	Died 3.7.14.

Discussion.

Some methods of treatment were apparently successful, but the word "apparently" is used advisedly, as in all cases where recovery occurred the method of treatment failed completely on more extensive trial. The only conclusion one could arrive at was, therefore, that the recoveries would have taken place even in the absence of any treatment.

The writer's experience with Lamziekte is that animals suffering from the disease should be left severely alone, as interference of any kind seems to hasten the paralysis and death of the animal. The cases which received medicinal treatment were either acute or subacute forms of the disease, no peracute or rapidly fatal cases being selected, so that the drug should have sufficient time to act. Only one conclusion could be drawn from the results of the experimental treatment discussed in this paper, and that is that not a single one of the different methods could be described as a successful cure for Lamziekte.

General Discussion and Conclusions.

A short discussion of the results obtained from the different experiments has been included in the remarks with which each experiment is concluded, and hence it is not thought necessary to review here again the different arguments for and against the various theories which have been advanced to explain the etiology of Lamziekte. It is perhaps more advisable to discuss very fully the conditions under which the disease is contracted.

Theiler (*l.c.*, p. 258) in 1912 reviewed at considerable length all the theories in connection with Lamziekte, and he himself brought forward the "Accumulative Vegetable Poison or Grass Toxin Theory," regarding which he concluded his remarks as follows:—"The accumulative poison theory explains the majority, if not all, of the observations in connection with Lamziekte." Since that time a great deal of work has been done on the other theories, such as the Avitaminosis theory, the mineral deficiency theory, and the Sarcosporidia theory. Experiments to test the Avitaminosis theory have been fully dealt with by Theiler, Green, and Viljoen, in the Third and Fourth Reports of the Director of Veterinary Research, published in 1915. The results of experiments carried out in connection with the mineral deficiency theory are fully reported on elsewhere in this paper; briefly stated, the results obtained afford strong evidence against a deficiency of minerals having anything to do with the etiology of Lamziekte. The Sarcosporidia theory, which was advanced by Professor Hedinger (*l.c.*, p. 297) is dealt with in a separate paper. After working at all these different theories for some time, we were forced to fall back on the "Grass Toxin" theory, which appears to be the only one capable of explaining all the observations which have been made in connection with the disease.* With regard to diseases in animals caused by a

* Since this article has been in the press further experiments were undertaken, which led to the definite conclusion that lamziekte is caused by the ingestion of bones and substances of certain carcasses undergoing putrefaction and containing a very active poison, which is probably of a specific nature and produced by a pathogenic saprophyte. Most cattle can be rendered lamziek by drenching with suitable material, when given in sufficient quantity (e.g. pupae of picrosoma as done in the experiments quoted in the article, the disease observed undoubtedly was lamziekte. Compare also second report of D. V. R., page 100, exp. 27; page 103, exp. 82; page 104, exp. 83; pages 175 and 176). Of cattle running in the veld only those animals contract the disease which show pica. In their searching for bones to satisfy their abnormal craving they pick up the toxic substance which frequently is contained in the bones. Practically all observations recorded in this article find now their explanation considering the fact that two conditions are required for the acquisition of lamziekte, viz. 1: pica, a morbid condition contracted from the pasture under certain climatic and telluric conditions, and 2: the poison of a toxogenic saprophyte, the development of which again is dependent upon climatic and probably also soil conditions as obtaining in lamziekte areas.

For details consult special report on the cause of Gallamziekte, by Sir A. Theiler, R. E. Montgomery, and P. R. Viljoen, etc., now in preparation.—A. Th.—May, 1919.

grass poison or toxin, a very interesting report by Jones and Arnold * has made its appearance quite recently. These authors have shown that a nervous disorder in sheep, called "Staggers," can be produced by feeding these animals on a coarse grass (*Poa argentina*). They conclude their report as follows:—(1) Staggers is a non-infectious disorder affecting horses, cattle, and sheep. (2) The disease is characterized by weakness, muscular twitching, irregular movement of the head, stiffness of the limbs, and transient motor paralysis, accompanied with spastic spasms on excitement. There is also derangement of vision and conjunctivitis. (3) The post-mortem lesions are not characteristic. (4) We readily produced the disease by feeding the susceptible sheep on a coarse tuft grass commonly known as coiron or pampa grass (*Poa argentina*). (5) The time required to produce definite symptoms by feeding the grass varied. Two animals developed typical Staggers after two feedings; in another instance a period of twenty-one days of feeding was required. The average time for the production of unmistakable symptoms was ten days. (6) Many sheep recover from Staggers spontaneously. A complete change of diet will usually effect a cure within two weeks. (7) Older animals which have pastured for long periods on lands where the grass grows become tolerant and are rarely affected with Staggers. (8) The grass is toxic to sheep at all seasons of the year. We fed late winter and early spring grass and grass in flower, and produced Staggers in every instance. The young green grass is as toxic as any edible portion of the plant." Elsewhere they state: "Attempts were made to extract some substances from the grass that would cause the symptoms. Acosta was unable to state definitely whether the plant itself contained an alkaloid or whether a toxin was produced by the action of a mould. The former seemed more plausible, as Patagonia is an exceedingly dry country. Moreover, the experiment in which we fed green grass which was not mouldy, seems to point to an alkaloid." The symptoms shown by cattle suffering from Staggers are not described, but those described in sheep differ in many respects from the symptoms of Lamziekte in cattle; whereas in the former disease recoveries are the rule, especially when the sick animals are given a complete change in diet, cases of the latter usually terminate fatally. The course of Staggers, as also its incidence, differs markedly from that of Lamziekte. Nevertheless, it is most interesting to find definite data regarding the toxicity of grasses in dry climates; and the further interesting fact is brought out that only one species of grass contained the toxic principle. That such a toxic substance can also be present on Lamziekte farms is shown by the discovery of a cyanophoric glucoside in the grass *Chloris petrea*, growing on Armoedsvlakte; the glucoside has so far not been detected in the same species of grass growing in the Pretoria District, a lamziekte-free area. As described elsewhere in this paper, a large number of grasses have been tested for the presence of this glucoside, but so far positive results have been obtained in the case of the one grass only. The symptoms produced by prussic acid differ widely from those seen in Lamziekte, and the disease in sheep caused by *Poa argentina* also does not resemble Lamziekte very closely, but the mere fact that these toxic principles

* Jones, F. S., and Arnold, J. F., "Staggers in Sheep in Patagonia," *The Journal of Experimental Medicine*, Vol. 26, No. 6, December, 1917.

may be present in certain grasses growing in dry countries is highly suggestive. Our knowledge of vegetable poisons is still a very limited one, and hence search for toxic substances by means of chemical analysis would be a most gigantic task. Lamziekte is essentially a disease of drought, occurring on grass veld in districts where the annual rainfall is a small one. Even on recognized lamziekte areas the disease disappears in good rainy seasons, as has been observed over and over again. The distribution of the rainfall during the summer months is also a very important factor; thus, fairly frequent rains commencing in early spring and continuing until the grass has become strong enough to withstand subsequent periods of drought seem to prevent the development of the disease. On the other hand, light showers of rain, just sufficient to stimulate the growth of vegetation, followed by long intervals of drought and a scorching sun, are conditions favourable for the development of the disease. Under these circumstances the growth of vegetation is repeatedly interrupted, the result being wilted and stunted grass. Grasses growing in shallow, sandy soil, over stony ridges, in veld that had been burned previously, owing to the lack of moisture in the soil, respond very quickly to unfavourable climatic conditions, and hence it is on such areas that the disease makes its appearance first. The statement is very often made that Lamziekte has been known to occur on veld in *perfect condition*, but it must be remembered that such good veld, in practically all cases, has been preceded by veld in very much worse condition. As has been shown in this paper, an "incubation" or latent period has been discovered in connection with Lamziekte, and hence whenever an outbreak of the disease occurs, one must take into consideration not only the veld conditions prevailing at the time, but also those existing several weeks before the outbreak. During the last few years the disease has made its appearance on farms where it was never known previously, and this apparent spread of the disease has led some farmers to conclude that Lamziekte is of an infectious nature, but this apparent spread of the disease can be easily explained by accepting the grass toxin theory. The general opinion amongst the older inhabitants of this country is that the country is "drying up," that on farms where in former years running streams were present no water can be found now near the surface of the soil. On questioning owners of farms, on which Lamziekte has made its appearance only recently, regarding the climatic conditions which prevailed in former years and those prevailing now, the immediate reply will invariably be that parts of the farm where formerly running streams and standing water were present, they are now able to place under cultivation. In fact, the history of the outbreak will always be that the disease first showed itself after a period of severe drought. With regard to veld conditions, the question is often asked why Lamziekte is so much less prevalent on native locations; the following answers are suggested:— (1) The natives were the first inhabitants of this country, and hence it would be quite reasonable to expect them to have selected the localities where their animals remained free from disease. (2) On the other hand, it is quite possible that after the long grazing down process to which native locations have been subjected, the veld conditions have been changed entirely, and that possibly the very grass or grasses, in which the toxin develops, have been replaced by other more healthy grasses. In our system of grazing down experiments the disease became even more prevalent after the veld had been grazed down, but these

experiments were not continued long enough for the grasses growing naturally in the experimental camps to have been replaced by other grasses. Probably if the grazing down process were continued for a number of years on the same area, this area would also become free from Lamziekte. In accepting a grass toxin as the cause of the disease, it still remains to be discussed whether this toxin is of a cumulative nature or not. The fact that moving animals from one part of a farm to another, or from one farm to another, checks an outbreak of Lamziekte for the time being points to the toxin being of a cumulative nature. So also does the fact that animals brought on to a lamziekte farm from a non-lamziekte farm do not contract the disease for some time after their arrival. The earliest period after which symptoms of the disease appeared in such animals was shown in another place in this paper to have been three and three-quarter months. Against the toxin being of a cumulative nature, stand the following observations:—(1) Mitchell had two cases of Lamziekte twelve and thirteen days, respectively, after the animals were muzzled, and during this period there could have been no further accumulation of toxin. (2) In experiment No. 32, described in another place, one animal showed symptoms of the disease twenty days after it was taken off the veld. (3) In the alternate feeding and grazing experiments (Nos. 23 and 24), the disease made its appearance in animals which had been kraaled and given a well-balanced ration for a certain period, during which no further accumulation of toxin could have taken place, and, in fact, one would expect that any accumulated poison present in the system of the animal would have been eliminated during the period of kraal feeding. It is more likely that the toxin takes longer to produce symptoms in one animal than in another, but one must admit that the length of time taken for the symptoms to develop in the Pretoria animals lends strong support to the toxin being of a cumulative nature.* As regards the true nature of the suspected grass toxin very little is known. What we know is that it only appears at certain times of the year when the grass is growing under unfavourable climatic and soil conditions. It does not seem probable that all grasses on a lamziekte area become toxic, because in that case one would expect the mortality to be much higher than it is even during the worst outbreaks. It appears to be more likely that the toxic substance is developed in only one, or perhaps in only a few grasses, but no doubt further experiments will throw light on this point. We have already planned experiments in which several plots of different species of grasses are to be established, species not only of Bechuanaland grasses, but also of grasses which do not grow naturally on the lamziekte areas. After such plots of grass have become properly established, a few animals could be allowed natural grazing on one species of grass only, but it stands to reason that the grazing would only be allowed when Lamziekte was prevalent on other parts of the farm. In this way it should be settled definitely whether or not the toxin could develop in any species of grass under the climatic and soil conditions which appear to have such a marked influence on the causation of this baffling disease.

* *Re* incubative periods in plant poisoning compare article on Senecio poisoning in this report by A. Theiler.

Preliminary Report on the Harmful Effects of "Steek" Grass on the General Health and Condition of Sheep.

BY

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THE term "Steek" grass (Dutch, *steken*—to prick or to pierce) is employed by farmers in South Africa to indicate any species of grass the fruits of which have sharp pointed ends that adhere to the fleece and pierce the skin of animals. Hence in various parts of the country, and even on different farms, grasses of different genera, such as *Aristida*, *Heteropogon*, etc., would be called "Steek" grass by the farmer. The term "Steek" grass is misleading, as it does not convey to the mind which part of the plant does the pricking. For many years past "Steek" grass has been known to adhere to the fleece of long-woolled sheep and to have a deteriorating effect on the wool, but it was only during the last year or so that serious attention has been drawn to this matter. It was only at the end of last summer that the writer was instructed by the Director of Veterinary Research to investigate and report on the harmful effects which "Steek" grasses were said to have on the health of sheep. This report, which should be considered as only a preliminary one, is based on observations made on Armoedsvlakte Veterinary Research Station, on different farms in the Vryburg District, and on the Government farm Bestersput, Orange Free State.

Species of Grasses Concerned.—The "Steek" grasses encountered during these investigations nearly all belong to the *Aristida* genus, the *A. congesta* being the most common species. *Heteropogon contortus*, which is said to be very prevalent in other parts of the country, was not encountered to any great extent on farms in the Vryburg area and will not be considered any further in this report. *Aristida congesta*, while young and green, is readily eaten by stock, but is avoided by practically all animals when it has reached the mature stage. At this stage the stems of these grasses become hard, fibrous, and unpalatable, and, as very little undergrowth or leaves are formed, they are not very tempting to animals. For this reason, to a great extent, *Aristida* will very often be found standing in pastures when most of the other more palatable grasses have disappeared. This point was very clearly brought out in some of our grazing-down experiments which were conducted in connection with investigations into the disease Lamziekte. In several small paddocks a comparatively large number of cattle and sheep were allowed to run for some months and, after the natural grazing in these paddocks was practically exhausted, the animals were still kept there and fed on artificial foodstuffs.

Grazing-down Experiment No. 1.—Into a small paddock, about 12 acres in extent, in which the grasses had already reached the flowering stage, 150 merino sheep were put for grazing. Within a period of three weeks the veld was practically grazed down and after that the sheep had to be given additional rations. The animals were kept on the same veld for another three weeks, during which time there was so little grazing that the animals had to live almost entirely on their additional rations. All the grasses on this small area, excepting *Aristida*, were grazed down completely, so much so that no species of grass other than *Aristida* could be recognized. From the beginning of the experiment the sheep avoided the *Aristida* species and this they continued to do right up to the completion of the experiment, in spite of the fact that the animals were continually roaming about in search of natural grazing. The result was that *Aristida* was the only grass which reached the fruit-forming stage. The fruits ripened and were afterwards shaken off the plant on to the ground; new grasses came up from the seeds and so converted practically the whole area into *Aristida* veld.

Grazing Down Experiment No. 2.—In a small paddock adjoining that in which the foregoing experiment was carried out, under exactly similar conditions, fifteen head of cattle were allowed to run for several months. In this case, however, the animals were placed in the paddock before the grasses had reached the flowering stage. The result was that all grasses, including *Aristida*, were eaten by the animals, and, as the grazing area was a very limited one, all the grasses were kept grazed down so short that none of them ever reached the flowering stage. Consequently *Aristida* did not get the upper hand over other species of grasses. It is a well-known fact that during the last few years grasses of the *Aristida* species have spread in many parts of this country, and this to such an extent that on some farms certain camps or paddocks are simply overrun by *Aristida*, almost to the complete exclusion of other species of grasses. It would be advisable, therefore, to discuss briefly the reasons for this enormous increase of *Aristida* grasses. The opinion in this country is almost unanimous that our annual rainfall in various parts of the country has been steadily decreasing for quite a number of years, and there is no doubt that it is largely due to the severe droughts that *Aristida*, being very hardy and drought resisting, has become such a predominant grass on many pastures where previously it was almost unknown. It has been a common observation in this part of the country that *Aristida* grasses always become predominant in the vicinity of new homesteads and water supplies, i.e. in places where there is an almost continuous congregation of and grazing down by animals. In this case again the prevalence of *Aristida* could be explained by the preference shown by animals for grasses other than *Aristida*, the result being that the latter remains standing, is allowed to reach maturity and to spread by the establishment of fresh shoots which come up from the seeds that were cast on to the ground. A further remarkable fact, which is difficult to explain, exists in that after new homesteads have been inhabited for some years the *Aristida* grasses tend to disappear, they being replaced by other species of grasses. This peculiar phenomenon can be explained, according to Mr. Pole Evans, Chief of the Division of Botany, by the different arrangement of the buds of various grasses. Thus, budding of some grasses takes place above ground, while

the buds of others are situated at varying depths under the surface of the soil. In the case of *Aristida congesta*, budding occurs from $\frac{1}{2}$ to 1 inch under the surface of the ground, whereas *Cynodon dactylon*, which is the grass succeeding *A. congesta* on homesteads, has its bud formation much deeper. The result is that the buds of *Aristida* are injured by close grazing and tramping down by animals, whereas the buds of *Cynodon*, on account of their deep underground position, are left undisturbed.

Dispersal of "Steek" Grass.—There is very little doubt that the wind plays a great part in the dispersal of grass seeds, including grasses of the *Aristida* species. Animals also carry the fruits of grasses from one part of the farm to another, although in the case of *Aristida congesta* this manner of dispersal could not be a very common one, as the fruits of *Aristida* adhere so closely and intimately to the fleece of the animals that they will only come off with the wool, and not before. It is possible, however, that some of the fruits may drop off before they have attached themselves properly to the animal's fleece.

Fruit of Aristida.—When a farmer speaks of "steek" grass he refers only to that part of the plant which does the pricking, namely, the ripe fruit. "Steek" grasses, therefore, only become troublesome to stock in the early part of winter, or as soon as the fruit has reached the mature stage. To understand the injury inflicted on animals by *Aristida* it is necessary to give a description of the mature fruit or "seed," the latter term being used synonymously with the word "fruit" in this article. The most important points in the structure of the fruit of *Aristida congesta*, as far as it concerns this report, are the following, namely:—(1) The comparatively long trifid awn above the lemma; (2) the sharp-pointed, hairy callus below the lemma. In the case of *A. congesta* the caryopsis is closely enveloped by a narrow, cylindrical lemma, which is indurated at maturity. The lemma extends below into a hard, sharp-pointed callus, which is in reality the elongated internode of the rachilla that remains attached to the floret. The callus bears numerous small hairs, which point in the reverse, or upward direction. On its upper extremity the lemma bears a trifid awn, which is comparatively long in the *Aristida*. These points are very clearly brought out in the accompanying drawings, for which the writer is much indebted to Mr. Pele Evans, Chief of the Division of Botany. Plate 1, Figure 1, shows the inflorescence of *A. congesta*. Figure 2 shows an individual spikelet: A, trifid awn; B, glumes; C, rachilla. Figure 3 shows the mature fruit: A, trifid awn; B, indurated cylindrical lemma, closely enveloping the caryopsis; C, sharp-pointed callus, armed with numerous retrorse hairs. Plate 2: Fruit of *Heteropogon contortus*, showing the long awn.

Mechanism for Attachment.—When walking through a field of ripe "steek" grass the wool of the animals is brought into close contact with the awns of the fruit, the result being that these awns attach themselves and adhere very intimately to the wool, bringing with them the whole fruit. Many of the fruits simply collect in the outer surface of the animal's fleece, and form themselves into large masses. Others, however, work their way up the wool fibres towards the skin, which they penetrate in many instances. This action is rendered possible by the peculiar structure of the callus, which, it will

be remembered, is provided with a sharp-pointed end, and armed with numerous retrorse hairs. As soon as the callus comes into contact with the wool fibres the tendency for it is to travel along the fibres towards the skin. The fruit is assisted in its upward journey along the wool fibres by the movements of the animal, and can only go in the one direction, as, by the action of the retrorse hairs on the callus, it is prevented from slipping backwards. The action described here is very similar to that of a barley ear, which can be made to travel up one's coat sleeve by placing it, with the awns pointing backward, at the entrance to the sleeve, and by alternately flexing and extending the arm.

Animals affected.—Merino sheep are the worst sufferers from "steek" grass, no doubt on account of their long wool and very tender skins. The wool of merino sheep consists of very fine, soft, rather curly and long fibres, which are very closely packed together. Persian and Africander sheep are said to be quite immune from the worries of "steek" grass. These animals have short and rather coarse wool, the fibres of which are smooth, straight, and not very closely packed together. On a few farms in the Bechuanaland area, where the *Aristida* grasses are particularly prevalent, the owners have given up farming with merino sheep, and have taken up the breeding of Persians and Africanders, with much success. On one of these farms during the winter months the writer inspected both types of sheep, and found the merinos to be badly infested with "steek" grass, and in miserable condition, whereas the Persians and Africanders were in good health and quite free from grass seeds. Generally speaking, it can be said that "steek" grass is troublesome to only long-woolled sheep, and that sheep with short wool are comparatively free from its worries. Other animals, such as cattle, goats, etc., experience practically no harmful effects from "steek" grasses.

Harmful Effects of "Steek" Grass.—The harmful effects of *Aristida* seeds on sheep may be considered under the following headlines:—(1) Effects on the wool; (2) effects on the skin; (3) effects on the eyes; (4) effects on the mouth; (5) effects on the general condition.

(1) *Effects on the Wool.*—It is especially the wool on those parts of the body which come into close contact with the grass seeds when the animal walks or grazes that is invaded to the greatest extent, namely, the wool around the legs, along the dewlap, sternum and belly, and around the head; the wool on other parts of the body may also be invaded to a smaller extent. In the sternal region in particular the grass seeds collect to an enormous extent, large masses of the seeds, very often up to the size of tennis balls, being formed in this position. In other regions also clusters of the grass seeds are formed, and the wool fibres are closely matted together. The grass fruits attach themselves very intimately to the wool fibres, so much so that in many cases it is quite impossible to detach these seeds from the wool without also pulling out the latter. The grass seeds, therefore, remain on the animals until the wool falls out or is clipped off. Although the presence of "steek" grass in the wool may not seriously inconvenience the animal, there is no doubt that it depreciates the market value of the wool to an enormous extent, chiefly by matting together the fibres, but also by actual loss of wool, which very often drops off in the sternal and abdominal regions.

(2) *Effects on the Skin*.—Fairly extensive pathological lesions are produced by "steek" grass in the skin of some animals. On account of the thick covering of wool the pathological lesions cannot be seen very clearly on the external surface of the skin, but in cases where the sharp-pointed callus of the fruit pierced the skin the lesions can be seen and studied on the internal surface, and in the subcutaneous tissues. On those parts of the skin which are devoid of wool or hair, however, the injuries caused by the pricking of the grass seeds can be seen in the very early stages. In these parts the lesion can be seen to commence as a small red spot at the seat of the injury, and to be in the nature of localized hyperaemia. Where the grass seed is removed early, and no infection has taken place, the lesion may not extend any further, in which case the small wound heals very rapidly. In many cases, however, the sharp-pointed callus not only remains in the skin puncture, but may even extend through the skin into the subcutaneous tissues, and into the fasciae covering the musculature. In most of these cases infection results from micro-organisms, usually cocci, which were present either on the grass seeds or on the external surface of the skin. Consequently small abscesses are formed subcutaneously, and in close relation to the sharp-pointed callus; in rare cases, where the seeds have penetrated into the musculature, abscesses form in this position. As a rule these small abscesses remain localized, excepting where several seeds have penetrated the skin in close proximity, when the abscesses formed in conjunction with each skin puncture would coalesce to form one large abscess. Although these small abscesses remain localized, the injury to the animal is very severe, especially when one remembers that hundreds of grass seeds may have pierced the skin of the same animal. If the hand is rubbed over the subcutaneous surface of the skin from an animal badly affected, the presence of numerous sharp-pointed seeds gives one the impression of a pin-cushion.

The symptoms shown by the animal are those of severe and continuous irritation of the skin; the animal almost constantly bites and scratches the irritating parts of the skin. The grass seeds always attack the animals during the winter months, and hence the cold weather combined with the constant skin irritation very soon reduces the condition of the affected animals to such an extent that many of them succumb to poverty and weakness. Many of the animals lose all their wool in the sternal and abdominal regions, the skin lesions being so extensive that the wool simply drops off, and brings with it large masses of "steek" grass.

(3) *Effects on the Eyes*.—Severe pathological lesions may be produced by the fruits of *Aristida* entering the eyes of sheep. It is especially those sheep which have a dense growth of long wool round the eyes that are affected, the wool serving as an anchor for the grass seeds. Clean-faced sheep, i.e. sheep which have no long wool on the face or round the eyes, seem to be comparatively free from attacks by "steek" grass. While the animal is grazing its face is brought into close contact with the fruits of *Aristida*, which attach themselves to the wool surrounding the eyes, and work their way into the cavity of the eye. The first symptoms to be noticed in the affected eye are severe lachrymation, with tears running down the face, and the eyelids partially or totally closed.

The eye lesions are caused by (a) the grass seeds themselves; (b) fly larvae, which are deposited in the tear-stained wool below the eye, and which enter the cavity of the eye. (a) The grass seeds, as a rule, lie free in the conjunctival sac, where severe conjunctivitis is set up, and, unless the foreign bodies are removed early, keratitis parenchymatosa will be the result. (b) The injuries produced by the fly larvae, however, are much more severe. As a result of lachrymation and soiling of the wool below the eye, flies are attracted to and deposit their eggs on the dirty, wet surface; larvae hatch out from these eggs, and enter the cavity of the eye, which is very soon destroyed, unless the larvae are removed almost immediately. These larvae, with the aid of pyogenic organisms, very soon invade the already inflamed tissues surrounding the eyeball, and soon after the eyeball itself it attacked, panophthalmia being the result. Unless a farmer is very careful to remove the foreign bodies from the eyes of his sheep many animals will lose the use of one or both eyes as the result of invasion by fly larvae. On account of the severe inflammation and pain in the affected eye, the loss in condition of animals suffering from ophthalmia is very marked. Fly larvae are very troublesome during the late summer months, and even after the cold weather has set in cases of severe ophthalmia resulting from larval invasion have been observed.

(4) *Effects on the Mouth.*—Mouth lesions, although not very common, are very serious when they do occur. The lesions consist of haemorrhagic dermatitis, which is produced by pricks received from the grass seeds which are present in the fleece of the animal. The animal constantly bites at the irritating places on its body, and in this way the skin round the mouth and nostrils receives pricks from the sharp-pointed callus of fruits that are present in the wool. At the commencement the lesions consist of small haemorrhagic spots scattered on the skin round the mouth and nostrils. By further pricks received in the way just described the haemorrhagic spots increase in number until they coalesce, when large open sores are formed. The size of these is further increased by the animal rubbing its sore mouth against such objects as stones, posts, etc., and in this way the whole skin surrounding the mouth and nostrils may become one large bleeding sore. In cases where treatment is neglected infection of the wound may take place, and serious gangrene of the skin may be the result. An animal suffering from extensive mouth lesions is unable to graze on the dry and fibrous grasses which are present on our veld during winter, and, unless special care is taken of such an animal—which should be given food of a soft and non-prickly nature—it will lose rapidly in condition, and may die of starvation.

(5) *Effects on the General Condition.*—General loss in condition is the worst and most harmful effect which “steek” grass can have on sheep. This was well illustrated on the Research Station at Armoedsvlakte where, in spite of plentiful grazing and comparative freedom from wire worm, the sheep had fallen off in condition tremendously. When one bears in mind that hundreds of grass seeds may be piercing the skin of an animal, one can understand that constant and severe irritation will be set up, and hence, apart from the question of pain suffered by the animal, its normal grazing and feeding will be greatly interfered with. This loss in condition of

sheep has been so general and so severe in Bechuanaland that many farmers have found farming with long-woolled sheep not a paying proposition, and have had to take up farming with short-woolled sheep.

PREVENTIVE TREATMENT.

To prevent the harmful effects of "steek" grass on sheep is chiefly a matter for the farmer to deal with, and is mainly one of careful management. The following, however, are some methods of prevention, by adopting which the trouble may be minimized to a large extent:—(1) Shearing and dipping; (2) Keeping sheep on veld free from "steek" grass; (3) mowing *Aristida* grass; (4) changing *Aristida* veld by cultivation; (5) close grazing and paddocking.

(1) *Shearing and Dipping*.—Experiments, which had for their object the freeing of sheep from grass seeds, and the prevention of further infestation, were carried out at the Armoedsvlakte Research Station during the last "steek" grass season. As long wool has been found to be favourable for the attachment of grass seeds, it was thought that by shearing the sheep the "steek" grass trouble would be greatly minimized. This was found to be the case, provided the shearing was carried out at the right time. The correct time for shearing can be arrived at only by studying the condition of the grass fruits. If shearing is resorted to before the fruits are quite ripe, i.e. before they are able to attach themselves to the fleece of sheep, the wool will have grown out again before the "steek" grass season is over, and hence grass seeds will enter the wool again late in winter when the sheep are in rather poor condition, and require all the nourishment they can possibly get. The best time for shearing has been found to be during the early winter months, or after the grass fruits have reached maturity, and have already started to invade the wool of the sheep. It is true that, to some extent, the value of such wool will be depreciated by the presence of the grass seeds, but this cannot be avoided, and, at any rate, the lives of many sheep will be saved by adopting this procedure. At the time when the sheep on Armoedsvlakte were shorn they were badly affected with "steek" grass, showing severe skin lesions and loss in condition. The shearing was followed soon after by dipping the animals in a lime and sulphur bath, the object being to find out whether the dipping fluid would hasten the decomposition of the fruits still remaining in the skin and wool of the animal. The dipping had a very beneficial effect, for, not only did rapid decomposition of the grass seeds take place, but also the skin lesions of the affected animals healed up quickly, so much so that within ten days after treatment no sign of "steek" grass, or the lesions produced by it, could be seen in the treated animals. After that the improvement in the condition of these animals was very marked, and, by the time their wool had grown fairly long again, the grass seeds had been shed on to the ground, and no further trouble was experienced with "steek" grass. An experiment on similar lines, excepting that dipping was not resorted to after shearing, was conducted at the writer's instigation by a prominent sheep farmer in the Bechuanaland area. In this case only some of the sheep were shorn, and towards the middle of winter there was a very marked difference in the condition of the shorn animals and those that had

not been shorn. The fleeces of the latter were full of grass seeds and their condition was rather poor, whereas in the shorn animals the fleeces were practically free from "steek" grass and the animals were in good condition. In this case shearing of the wool, even when not followed by dipping, seems to have had the desired results, although there can be no question of the further benefit to be derived from the dipping fluid.

(2) *Keeping Sheep on Veld Free from "Steek" Grass.*—On most farms in this country *Aristida* is confined to certain definite areas or patches, and hence, by careful farm management, it is quite possible to keep the animals away from such areas. On some farms *Aristida* grasses are plentiful near the homestead or waterholes, and hence in these cases it is necessary to cut a broad path through the belt of *Aristida*.

(3) *Mowing Aristida Grass.*—Although the mature plant has very little nutritive value, *Aristida* is well eaten by stock when it is young and green. Where the grazing on a farm consists largely of *Aristida* veld, it may be necessary to mow down large areas of such grasses before they have reached the flowering stage, so as to render the veld safe for long-woolled sheep. The cut grasses could then be made into hay and stored for winter feeding, or else they could be used as bedding for animals.

(4) *Changing Aristida Veld by Cultivation.*—Close observers are well acquainted with the fact that by cultivating virgin soil, or even by only ploughing and leaving it fallow, the vegetation on such soil will be changed completely. By cultivation, therefore, it is quite possible to convert *Aristida* veld into veld in which grasses of other genera predominate. This method of exterminating *Aristida* grasses should be applied on farms where "steek" grass is very prevalent, and where it is difficult to carry out the other methods of preventive treatment enumerated in this report. On the farm Besterput, in the Free State, the change of vegetation after ploughing was well shown in one camp where *Aristida* was the predominant grass. The ploughed field is still surrounded by *Aristida*, but on the ploughed area itself *Aristida* has been replaced almost entirely by other kinds of grasses, mostly *eragrostis*.

(5) *Close Grazing and Paddocking.*—One of the reasons for the failure of sheep farming in the Bechuanaland area is that the farms are much too large, not fenced off in camps or paddocks, and understocked, the result being that the grasses grow tall and very rank, and that the farmer cannot keep proper control over his stock and his grazing areas.

Experiments have shown clearly that close grazing over *Aristida* veld which has already reached the mature stage will tend to increase the *Aristida* grasses on such veld, but, on the other hand, if overstocking is resorted to on *Aristida* veld when the grasses are young and green, the animals will eat the *Aristida* grasses, and so prevent them from maturing and spreading. In cases, therefore, where a farm is overrun with "steek" grass, it is recommended that it be fenced off into paddocks, and that these be grazed down in rotation by a large number of animals so that the grasses in the paddocks would not reach the fruit-bearing stage. If the grasses have already reached the flowering stage, cattle should be put in the paddocks to tramp

down the grass stems, and so cause the fruits to be cast on to the ground.

CURATIVE TREATMENT.

This does not play any great part in the treatment of pathological lesions produced by the seeds of *Aristida*, as the most common and widespread lesions, namely, those affecting the skin, would heal and disappear rapidly as soon as the cause is removed. Immersion of the affected animals in the ordinary sheep dips, especially the lime and sulphur dipping fluid, would tend to hasten the healing process. The treatment of lesions involving the eye and the mouth is made easy by the fact that these lesions occur only in a small minority of animals. Treatment of the eye lesions should be commenced early, first by clipping short the wool round the eye, and, after removing the grass seeds from the conjunctival sac, by the application of simple eye lotions, such as boracic acid, silver nitrate, etc. If the animal's face is kept clean, and matting of wool below the eye is prevented, not much trouble will be experienced with fly larvae. With regard to the mouth lesions, by removing the skin irritation on other parts of the body, i.e. by shearing and dipping the animal, these lesions will respond speedily to the application of an ordinary zinc ointment. While under treatment the animal should be fed on green and soft foodstuffs, and be kept away from coarse grasses or other forage, so as to avoid further injury to the mouth.

CONCLUSIONS.

1. "Steek" grasses cause serious injury to the general health and condition of long-woolled, especially merino, sheep.

2. Pathological lesions, caused by the penetration of the sharp-pointed grass seeds, are produced in the skin covering the body, round the mouth and nostrils, and in the eyes of merino sheep.

3. *Aristida congesta* is the grass which does the most harm to sheep in the Vryburg District, although other grasses, species of *Aristida*, and *Heteropogon contortus*, have also been found to cause injury to animals.

4. The mature fruit or seed is the part of the plant which causes the injury, and hence "steek" grasses become troublesome only during the winter months.

5. During the last few years *Aristida congesta* has been on the increase on many farms in the Vryburg District, and the methods by which the grass is spread are discussed fully in this article.

6. The harmful effects of "steek" grass on sheep can be prevented, to a great extent, by careful farm management, and by adopting one or more of the preventive measures which are discussed in this report.

7. Experiments carried out at the Armoedsvlakte Research Station have proved that the harmful effects of "steek" grass can be minimized, to a great extent, by shearing and dipping merino sheep during the early winter months.

Plate 1.

Fig. I.

INFLORESCENCE
(NAT. SIZE.)

a

Fig. III.

b

FRUIT. X. 4.

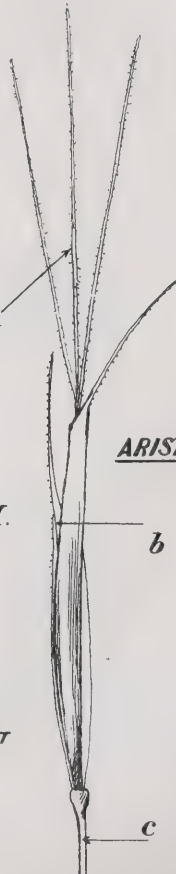
c



a

Fig. II.

b

SPIKELET
X. 4.ARISTIDA CONJESTA R. & S.H. LANSDALL,
DIVISION OF BOTANY.
PRETORIA.

c

Plate II.*HETEROPOGON CONTORTUS* R & S.

H. LANSDELL,
DIVISION OF BOTANY,
PRETORIA.

FRUIT. x. 2.

Contagious Abortion of Cattle in South Africa.

BY

E. M. ROBINSON,

Veterinary Research Officer, Onderstepoort.

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THERE has not been a great deal of literature published about Contagious Abortion during the last three or four years. The following papers contain a good deal of interesting information bearing on the subject:—

A long paper on "The Alterations in the Uterus in Epizootic Abortion and in some other Infectious Metrites in Cows," by Sven Wall, Stockholm, was published in the Report of the Tenth International Veterinary Congress, 1914. This paper is devoted entirely to pathology, and the general conclusion is that the actual changes caused by the abortion bacillus are not of a very serious character in themselves, and the uterus recovers with slight atrophy and sclerosis of the mucous membrane. It is only when secondary infections, such as pyogenic and mixed infections of different types of bacteria, occur that serious changes take place in the uterine mucous membrane with resulting atrophy and sclerosis, and ending in the sterility of the cow. The streptococcus pyogenes infection appears to be the most serious, and causes the most marked changes, resulting in a chronic metritis.

Three other papers on Contagious Abortion occur in the Report of the Tenth International Congress, 1914—two general ones by Professor Moussu, of Alfort, and Dr. Zwick, of Vienna, respectively, and one on artificial immunization in Contagious Abortion in cattle by Sir Stewart Stockman. The latter paper gives details of the inoculation of a large number of cows and heifers with both living and dead cultures of *B. abortus*, and concludes that the highest degree of immunity that can be conferred is that given by massive doses of living cultures given subcutaneously. Only non-pregnant heifers or cows are inoculated before being put to the bull, and the results obtained are considerably better than those obtained by any other method.

The Annual Report of the New York State Veterinary College for 1913-14 contains an article on Contagious Abortion by W. Williams, in which a good many statements are made which appear to be too dogmatic. He states that retained afterbirth is always due to metritis due to Contagious Abortion.

Although this is undoubtedly true for the majority of cases, it is nevertheless a fact that other forms of metritis may occur during gestation and lead to retention of the afterbirth. Another statement, that about half the cows which abort will not react to serum tests at the crisis of the disease, but will if followed up for 20 to 30 days after abortion, does not appear to be true in South Africa, and of the cows' sera which have been tested after abortion all have given a reaction in a dilution of at least 1-100. From a few cows which had aborted and given negative agglutination tests at the date of abortion, samples of serum were taken weekly for a month after abortion, but with negative results. These negative cows were not considered to be infected with Contagious Abortion, and a satisfactory explanation could usually be

found, as several of the stock diseases of South Africa have abortion as an occasional symptom. White scour in calves has not been associated here with Contagious Abortion, but as the conditions are usually ranching ones, the disease has not much opportunity of spreading. Williams makes the statement that Contagious Abortion is universal in bred animals and common in virgin heifers. As yet this does not appear to be the case in South Africa, though the disease has a wide distribution. Williams believes that infection of a cow can only occur through the unsealed cervical canal of the uterus, and that if infection does occur by ingestion, the bacilli go through the alimentary tract and get to the uterus by faeces from the anus soiling the vulva and vagina.

A further article on the subject of Contagious Abortion occurs in the Report of the New York State Veterinary College for 1914-1915 again by W. Williams. A large part of the article is devoted to the granular venereal disease in cattle. An interesting series of cases is mentioned of calves brought up on boiled and raw milk respectively. Those brought up on raw milk are stated to always show staining and matting of hair on the prepuce in the case of bulls, and the hair at the lower end of the vulva in heifers. This staining is from a slight discharge of blood-stained mucous material, and calves brought up on boiled milk are stated not to show any staining of the sexual hairs.

A most interesting article has appeared recently in the *Journal of Agricultural Research*, Vol. 9, No. 1, 4th April, 1917, by Schroeder and Cotton on "Some Facts about Abortion Disease." The following statements are made in this article:—"The favourite habitat of *B. abortus* in the non-pregnant cow is the udder and it may remain infected for as long a time as seven years. Milk from aborting cows and infected cows which do not abort, is infected continuously in some cases and in others intermittently. When the milk of a cow which gives a positive serum reaction in both its serum and milk, shows absence of abortion bacilli, the serum reaction will be found to be gradually lost, if the history is followed up. This supports the idea that the persistence of agglutinins in the blood is due to continual infection from the udder via the lymphatics. Abortion bacilli do not maintain themselves in the body of a cow except in the udder and pregnant uterus. Abortion bacilli inoculated into the blood of a normal cow, non-pregnant, disappear from the blood-stream in a few hours, and if the cow be killed later, bacilli will only be found in the udder and its lymphatics. In one case of a virgin heifer the bacilli made a lodgment in the non-functional udder. Several artificially infected cows were killed and portions of all the internal organs, glands, brain and nervous system, synovia, etc., were inoculated into guinea-pigs without result except in the case of the udder, which always showed one or more infected quarters, one or both supra-mammary glands, and in one case the pelvic glands, being infected. Abortion bacilli inoculated into the non-pregnant uterus disappear in a few days, and in the uterine discharge after abortion cannot be found after 50 or 60 days. Cows with infected udders, whether they have aborted or not, may give birth to seemingly normal calves in a normal manner associated with abortion bacilli in the uterus and afterbirth. This may occur at the third calving after abortion. This fact was never observed in cows with a positive agglutination reaction, but no bacilli in the milk. Tests of the uteri of non-pregnant cows,

before, during, and after oestrus, were negative. Bodies of newborn calves from cows with infected udders were found to harbour abortion bacilli in the stomach, gastrohepatic glands, etc., but only when the uterus was infected."

All these facts are of much interest and are based on the inoculation of guinea-pigs. The possibility of its requiring a considerable number of abortion bacilli to set up lesions in a guinea-pig has, however, not been taken note of, and it may yet be found that other centres of infection exist in cattle, such as the alimentary tract or some part of it.

In the only case here where a guinea-pig survived inoculation intraperitoneally of a small quantity of faeces from a badly infected cow, a negative result was obtained, but such a result cannot be considered of much importance. According to observations here, the virulence of different strains of *B. abortus* varies very much, in a few cases only the positive agglutination titre of the guinea-pigs' serum and subsequent histological examination of the spleen, allowing one to make a definite diagnosis. The history of a case of artificial infection of a pregnant cow via the teat canal is given by Schroeder and Cotton in the same article. Infection of the uterus occurred, but the conditions of the test probably do not occur naturally, as the dose given was large. Infection via the teat canal can undoubtedly occur, but it would not appear to be frequent, and under South African conditions must be considered rare, particularly on ranches, where cattle are not in one place for long. Probably the most interesting feature of this article is a report of two cases where bulls with positively agglutinating sera come under examination. One with an agglutinating titre of 1-200 had an abscess of the epididymis containing abortion bacilli. Other parts of the genital tract were negative. The other with an agglutination titre of 1-100 showed abortion bacilli in the semen collected from the vagina of a clean cow after the bull had served her. Urethral infection was not excluded here, but the cow would not appear to be infected as all tests of her were negative.

An article by Reinhardt and Gauss on "The Antibodies which occur in the Blood and Milk of Animals affected with Contagious Abortion" appeared in the *Zeitschrift für Infektionskr. Parasitäre Krankheiten u. Hygiene*, Vol. 26, No. 4, 13/1/15. This article is devoted to comparative tests of the blood serum and milk of infected animals, and concludes that with a titre of 1 : 20, a milk must be considered positive. Milk-whey, made by using rennet, was employed in the tests.

Professor Zwick, Vienna, has an article in the *Berliner Tierärztliche Wochenschrift* for 16/1/13, Vol. 19, No. 3, on "The Excretion of Abortion Bacilli in the Milk of Infected Animals."

Goats were used in the experiment quoted. These goats had normal udders and one was inoculated with a pure culture intravenously, one subcutaneously, and the third by the intramammary method. The one which received the intramammary infection was only inoculated into the right half of the udder, and excreted bacilli from this half for three to five months, no bacilli being obtained from the left half. The one dosed intravenously excreted abortion bacilli in the milk for two months, during which it was followed up, the

infection commencing 24 hours after inoculation. The goat done subcutaneously only started excreting bacilli in the milk after two days and ceased after six days.

Several articles have appeared in the *Journal of Comparative Pathology and Therapeutics*. In the number for June, 1913, Vol. 26, Part 2, is an article on "Researches regarding Epizootic Abortion in Cattle" by Sir John MacFadyean, A. L. Sheather, and F. C. Minett. The paper is devoted to the tests of the sera of cattle infected artificially by virulent cultures, given subcutaneously, intravenously, by mouth, by the vagina in cows and by the prepuce in male cattle. The results show that after infection, if positive, an animal usually gives positive serum reactions within three weeks, and if not positive before the third week the injection has probably failed.

The number for March, 1915, contains a paper on "Some Observations on the Methods of using the Agglutination Test in the Diagnosis of the Disease in Bovines caused by the Bacillus of Contagious Abortion," by H. R. Seddon. This paper is mainly devoted to a description of the various sources of possible errors which may occur in conducting agglutination tests. The agglutination test is suggested as a means of diagnosing the presence of *B. abortus* in a mixed infection of material such as uterine exudate by inoculation of guinea-pigs, the serum of guinea-pigs to be tested at intervals after the inoculation. In one of his cases, where a guinea-pig was killed about two or three months after inoculation, an enlarged spleen was found, and was undoubtedly due to the infection, though the author lays no stress on it and did not use it for cultural or histological examination.

In the number for December, 1915, L. E. W. Bevan has an article on "A Simple Method of obtaining Serum for the Agglutination Test for Contagious Abortion." This paper gives a description of a method of collecting samples of serum in countries such as Rhodesia, where a test may not be possible until a week or two after taking, on account of transport difficulties. Wright's pipettes are used, containing a mixture of boracic acid, sodium chloride, and distilled water, 3:9:100, and blood is added in the proportion of 5:1 of the mixture. Bevan had considerable success with this method after trying various others.

INTRODUCTION.

The existence of Contagious Abortion amongst cattle in South Africa was definitely proved for the first time in 1913. Until then, though the presence of the disease was generally admitted, and it had caused considerable losses to stock-owners during many years, it had not been settled beyond doubt that the disease was due to the *Bacillus abortus* of Bang. References to abortion in cows, presumed in the absence of proof to be Contagious Abortion, have been made from time to time in the *Agricultural Journals* of the Cape of Good Hope and Transvaal and in the Annual Reports of the Chief Veterinary Surgeons for these two Provinces. A popular bulletin by C. E. Gray, Chief Veterinary Surgeon of the Transvaal, was written for distribution amongst farmers (*Agricultural Bulletin* No. 6, 1906). In this he refers to a severe outbreak of Contagious Abortion in the vicinity of Johannesburg, which was traced to the introduction of an infected cow into the herd. The article was mainly devoted to preventive measures and treatment, and a full description of such measures

was given. The number of outbreaks which occurred during the year ending March, 1916, was 26, and for the year ending March, 1917, 42. Outbreaks have only been recorded since the introduction of the agglutination test in 1914 as a means of diagnosing the disease. It is only of late, since suspected herds in the Union have been subjected to the serological tests, that it has been seen how very widespread the disease is. Positive results have been obtained from most parts of the Transvaal, Orange Free State, and the Cape Province, particularly the Western Province. Natal and Swaziland have infected farms, losses being very heavy in both districts. Not only is the Union affected, but Southern Rhodesia has infected farms, as reported by Bevan, and the disease undoubtedly exists in both North-Eastern and North-Western Rhodesia. Sera sent from Livingstone have proved positive when tested at this Laboratory, and Mr. H. E. Hornby, formerly a veterinary surgeon under the Northern Rhodesian Administration, assures me that abortion of a type that swept through herds occurred in his districts occasionally, leaving little doubt in his mind as to its infectious nature. It is very probable that all parts of the continent of Africa into which imported cattle have been introduced contain infected cattle, as there is no restriction on the exporting of infected cattle from Europe, and no countries as yet require cattle to be subjected to serological tests before importing them.

During 1913, Mr. G. N. Hall was successful in establishing the identity of the South African and European diseases. He obtained a pure culture of the *B. abortus* from the stomach of a foetus which had been obtained from a suspected farm and he was able to prove its morphological and cultural identity with the European strains. Cultures were obtained from Stockman in England and Zwick in Germany. These European strains and a South African one were agglutinated to the same degree by a positive serum. He found that in the majority of cows which aborted the sera would agglutinate in a dilution of 1-800, less often 1-500, and rarely 1-400. The total number of cows tested, aborted and normal, was about 200. When tested with a positive serum, the South African strain was found to be agglutinated to a slightly higher degree than the European ones. The highest dilution in which a water-clear agglutination was obtained with any serum was 1-2000. This work was done between May and August, 1913, and on Mr. Hall's departure the work was handed over to Mr. Viljoen, who did a few further experiments. He fixed the standard at, or over, which a serum was to be considered positive, at water-clear agglutination in a 1-100 dilution. This standard is higher than the one used by European workers, which is agglutination in a 1-50 dilution. Tests were undertaken which confirmed the previous work on the comparison of the South African and European strains. Standard emulsions in .5 per cent. carbolyzed physiological water were found to agglutinate better when fresh than after they had been stored for fifteen days in the ice-chest. A few tests were undertaken to see whether sera which had been kept for at least a month and even become contaminated, still retained their agglutinating power to the same degree. No preservative was added to the sera, and when tested they were found to have retained their titre which they had when fresh. This observation is in accordance with those of a great many observers, and it is an accepted fact that the stability of agglutinins is very great. The work was handed over to Dr. Veglia of this institute in November, 1913, and he carried

out numerous tests on sera from cattle in the outlying districts. In July, 1914, the author took over the work, since when numerous experiments have been done and tests on the sera of upwards of 2000 cattle from the field have been undertaken. After trying several methods of collecting sera in the field, the present system, which has proved satisfactory, was adopted. It consists simply of sending out small sterilized bottles of 20 c.c. capacity and having the blood collected in them. These bottles, if dispatched immediately, generally arrive with the serum which has been expressed, quite clear. The bottles generally arrive within a day or two of filling, so that contamination with staining of the serum as a result is never marked. Occasionally the serum is not expressed from the clot, but this might occur with any method and does not present a serious problem. The existence of Contagious Abortion has now been proved in a great many districts of South Africa. It must originally have been imported into South Africa with European cattle, and infected cattle have probably been imported from time to time and are still coming in. Up to the present, no methods of immunizing against the disease have been introduced into the Union of South Africa, and it is not proposed at present to attempt them. The reason is that at present the disease, although widespread, is not considered beyond control by means of quarantine, when its presence has been established on a farm. Methods of immunization will not be undertaken until other methods of control fail, and it is hoped that judicious quarantine measures will reduce the spread. Infection is probably spread as much by the bull on the large ranches in South Africa as by other methods. Ingestion is still an important factor, but probably does not play such an important part as it does in dairy farms and places where the cattle are kept close together. Much difficulty has been experienced in determining by serological tests whether a bull is infected or not. Positive reactions have occasionally been obtained from bulls of infected herds, but negative results are the rule. We do not advise the use of a bull from an infected herd for clean cows, even when giving a negative reaction, as it seems certain that many such bulls are infected in the prepuce only, not giving cause to agglutinin production in the body. Again, bulls which give a positive reaction are not necessarily infected in the prepuce, so that the question of infection in bulls is a difficult one to settle. The agglutination test has been used chiefly in the diagnosis of Contagious Abortion, but the complement fixation test has been used as well as a control in a few doubtful cases. The method used in the agglutination test is that used by most European workers, and it has been described so often, descriptions of it appearing in almost every paper on this disease, that a description here would be superfluous. In a few cases the non-occurrence of agglutination in the stronger dilutions of a strongly positive serum has been noticed. This is a common observation on agglutinating sera, and the actual cause of it has not been definitely settled. In the tests undertaken here it was never found to occur with a fresh serum, but almost invariably with one which had been kept for a few days, even in the ice-chest. In one case it occurred with a serum which, on the day after taking, agglutinated distinctly in every dilution up to 1-1000. After a week at room temperature, though still clear, it would not agglutinate in any dilution below 1-100. Finally, this serum lost all power of agglutinating, the highest dilutions being the last to fail. The positive standard fixed at 1-100

by Viljoen was retained for sera from the field where an opportunity of getting samples of serum later might not occur, and proved to be a fairly safe standard for such use. In the case of infected herds, however, where one could obtain samples easily, it was found that even agglutination in a dilution of 1-25 could not be regarded as free from suspicion, and in numerous cases the sera of cows which gave an agglutination of 1-25 or 1-50, gave, a month or two months later, an agglutination of 1-200 or over. This point was rather forcibly brought home here by the case of one of the cows in our infected herd, which, when tested for the first time, gave an agglutination of 1-25, and was therefore considered as negative at the time. Three weeks after the test she had a typical abortion with retained afterbirth and yellow necrotic cotyledons, a yellowish brown creamy discharge being present in the uterus. At the date of abortion the serum gave water-clear agglutination in a dilution of 1-200. The point was further brought out by the experiment conducted to attempt the cleaning up of an infected herd by means of agglutination tests at intervals of two months (see Experiment No. 1). In that experiment several cows might have been passed as negative on one test. Our present system is to undertake, where possible, more than one test of an infected herd. More importance is attached to those cattle giving negative results at a first test, and a second test of them is always advised. Those positive at first test are isolated, and retested if possible, but most importance is attached to the retesting of those previously giving negative results.

EXPERIMENT No. 1.

Experiment to arrest the spread of Contagious Abortion in a herd by separating the cows that give a positive reaction to the agglutination test.

This experiment was undertaken with the object of completely freeing an infected herd from Contagious Abortion by removing all cattle that showed positive reactions to the agglutination test, the sera of the whole herd being tested at certain intervals. Sixty-three cattle—cows, heifers, and a bull—from a herd in which Contagious Abortion had existed for some time, were sent to a clean farm, that is to say, one on which Contagious Abortion did not exist, nor did it on any of the neighbouring farms. Previous to the removal in August, 1915, the whole herd had given negative reactions with one exception: a cow which had calved normally in 1913, and was not in calf on arrival on the new farm. The serum of this cow, No. 1148, had given a positive agglutination in January, 1915, in a dilution of 1-200. In August, 1915, the agglutination test of her serum gave a positive result in a 1-1000 dilution. This cow was accidentally included in the herd, but was taken out at the first test on the clean farm. The first test of the whole herd on the clean farm was carried out in January, 1916, and five reactors in a dilution of 1-100 or over were obtained. The numbers of these were 1148, 3103, 3080, 3335, and 3329 (see table). They were removed and

isolated a fortnight after being bled for testing purposes. The subsequent history of these five cows is as follows:—

Cow No. 1148.—Calved on 28th August, 1916. The calf was big and strong, and the foetal membranes came away normally. This cow, nevertheless, showed abortion bacilli in the milk, guinea-pig inoculations giving positive results. She calved again on 26th December, 1917, in a normal manner, the milk again proving to be infected with abortion bacilli, as shown by guinea-pig inoculation. The after-birth was infected as well as the milk at the second calving, and was probably infected at the first, but tests of it were not then made.

Cow No. 3103.—Aborted on 11th November, 1916. The foetus could not be found. Inoculation of the milk into guinea-pigs gave positive results, therefore the cow must have been harbouring abortion bacilli in the milk.

Cow No. 3335.—This cow aborted on 13th December, 1917, two years after isolation from the herd. She had been kept isolated with other reacting cows, and had not calved in the interval, although she had had access to a bull. Her milk proved positive on inoculation into guinea-pigs.

Cow No. 3080.—Has not calved since separation from the herd.

Cow No. 3329.—Calved in a normal manner without retention of the afterbirth on 25th February, 1917, the calf being strong and active, and not small in size. The cow's milk, however, gave a positive result on inoculation into guinea-pigs. The afterbirth was not obtained, so that it is impossible to give a definite opinion as to whether the uterus was infected or not.

The second test of the complete herd two months later revealed the presence of three further reactors, cows Nos. 3307, 3380, and 2737. These three were immediately isolated with the previous five in a separate camp on the farm to which none of the other cattle had access.

Cow No. 3307.—Has not calved since isolation.

Cow No. 3380.—Calved normally on 4th November, 1916. There was no suspicion of abortion, and the cow may be regarded as having calved in a normal manner. Guinea-pig inoculation of milk was not done in this case.

Cow No. 2737.—Aborted in June, 1916. The agglutination of this cow's serum was 1-2000 at the date of abortion. The foetus was not obtained. The abortion occurred in the separate camp of the farm in which the cow had been isolated with the other reactors. The cow was quite dry on arrival at Onderstepoort a week after aborting, so that no milk could be obtained for guinea-pig inoculation. It was decided in future to bring all the reacting cattle to Onderstepoort before calving had taken place, to ensure material for guinea-pig inoculation. All the reactors were brought up to this Laboratory for observation after the cow No. 2737 had aborted, and were no longer isolated on the farm itself as they previously had been.

The third test was made in May, 1916, and there was only one positive reactor, cow No. 3403.

Cow No. 3403.—Aborted on 22nd July, 1916, the *Bacillus abortus* being obtained in pure culture from the milk. The foetus was at

about the seventh month of pregnancy and retention of the afterbirth occurred. The abortion occurred at Onderstepoort, the cow having been brought up in June, 1916, with the other reactors. The cow again aborted on 14th December, 1917, the foetus this time being fully developed. Retention of the afterbirth again occurred, and the milk of the cow, emulsions of afterbirth, and stomach contents of the foetus proved infective for guinea-pigs.

The fourth test was carried out in July, 1916, and two further reactors were found—cows Nos. 3201 and 3334, both of which were immediately brought to Onderstepoort.

Cow No. 3201.—Calved apparently normally on 6th October, 1916. The calf was small and weak, but lived. *B. abortus* was obtained in pure culture from the milk of the cow. The calf was probably premature, but no retention of the afterbirth of the cow occurred.

Cow No. 3334.—Calved on 19th September, 1916. The calf, though alive when born, could not stand up or suck, and was very small though well formed. It appeared to be at about the seventh month of gestation, and was killed for culture making. No cultures of *B. abortus* were obtained from the calf, but were obtained from the milk of the cow. The cow again aborted on 16th August, 1917, the foetus this time being at the third or fourth month of gestation. Guinea-pig inoculations from the cow's milk, afterbirth, and the stomach contents of the foetus proved positive.

Subsequent History of the Herd.

The cows still remaining were all sent to another clean farm (Farm A). On 17th August, 1916, the fifth test was made, and there were no further positive reactors. In this experiment a bull, No. 2600, was running with the herd of cows, but at no time did he give an agglutination of even 1-10. This does not exclude the possibility of his being infected at least to a slight degree in the prepuce, but as he had not served a cow which had aborted since August, 1915, and only a few with positively agglutinating sera, one may presume that he was probably not infected.

The herd from which the sixty-three cows and bull were taken in August, 1915, had been an infected one for a year previously. It had originally consisted of about 100 cows, heifers, bulls, and a few oxen. Ten actual abortions took place in it previous to August, 1915, and sixteen other cows were positive reactors to the agglutination test. The herd had been running on a farm (C), and in August, 1915, the aborters and positive reactors were brought to Onderstepoort, the sixty-three cows and bull used in this experiment being those left over as non-reactors.

At the present date, two years after the last test of the herd after removal to Farm A, no further reactions have appeared, and none amongst the clean herd into which they were introduced.

Conclusion.—This experiment shows that by means of agglutination tests a herd can be cleaned from infected cattle, if the tests be undertaken at definite intervals, and cows giving a positive agglutination test are removed immediately.

ISOLATION EXPERIMENT.

Cow.	27.12.15.	3.3.16.	12.5.16.	4.7.16.	7.8.16.	
3120	—	+ 1-10	—	—	—	Isolated.
3380	+ 1-50	+ 1-100	—	—	—	
3398	—	Dead	—	—	—	
3254	—	—	—	+ 1-10	—	
960	—	—	—	+ 1-10	—	
2598	—	—	—	—	—	
3297	—	+ 1-10	—	+ 1-10	—	
3003	+ 1-10	—	+ 1-10	—	—	
3222	+ 1-10	+ 1-10	—	—	—	
3376	+ 1-10	—	+ 1-10	—	+ 1-10	
2947	—	—	—	—	—	Isolated.
1148	{ + 1-400 ± 1-800	—	—	—	—	
3057	—	Dead	—	—	+ 1-10	
1937	—	—	—	—	—	
3201	+ 1-10	{ + 1-25 ± 1-50	Isolated temporarily	+ 1-200	—	Isolated.
3091	+ 1-10	+ 1-10	+ 1-10	+ 1-10	+ 1-10	
570	+ 1-10	Dead	—	—	—	
3103	+ 1-800	—	—	—	—	Isolated.
2581	—	+ 1-25	+ 1-50	+ 1-50	{ + 1-25 ± 1-50	
3329	+ 1-1000	—	—	—	—	Isolated.
3332	—	Dead	—	—	—	
2667	—	+ 1-10	+ 1-10	+ 1-10	+ 1-10	Isolated.
1188	—	—	—	—	—	
3334	+ 1-25	+ 1-10	+ 1-25	{ + 1-500 ± 1-800	—	
1149	—	—	+ 1-10	+ 1-10	+ 1-10	
2658	+ 1-10	—	—	—	+ 1-10	
2672	+ 1-10	Dead	—	—	—	
2752	+ 1-10	—	—	—	—	
3396	—	—	—	—	—	
2707	+ 1-10	—	—	—	—	
3004	—	—	—	—	—	
3416	+ 1-10	—	Dead	—	—	Isolated.
643	—	—	—	—	—	
3333	+ 1-25	+ 1-25	+ 1-25	+ 1-50	+ 1-50	
2737	+ 1-10	+ 1-200	—	—	—	
3247	—	—	—	—	—	
3307	—	+ 1-200	—	—	—	
3059	+ 1-10	—	—	—	—	
3306	+ 1-10	+ 1-10	+ 1-10	—	—	
2756	+ 1-10	—	—	{ + 1-10 ± 1-25 }	+ 1-10	
2790	—	—	—	—	—	
3403	—	—	{ + 1-50 ± 1-100 }	—	—	Isolated.
3031	—	+ 1-50	—	—	—	
Bull	—	—	—	—	—	
2600	—	—	—	—	—	
Cow	—	—	—	—	—	
2953	+ 1-10	—	—	—	—	
3100	+ 1-25	+ 1-25	Dead	—	—	
2579	—	—	—	—	—	
3381	—	—	—	—	—	
938	—	—	—	—	—	
3348	—	—	+ 1-10	—	+ 1-25	Isolated.
3256	—	+ 1-10	+ 1-10	—	+ 1-10	

ISOLATION EXPERIMENT—(continued).

Cow.	27.12.15.	3.3.16.	12.5.16.	4.7.16.	7.8.16.	
2746	---	---	+ 1-10	---	---	
3428	---	---	---	---	---	
2567	---	---	---	---	---	
2584	---	---	---	---	---	
3097	---	---	---	---	+ 1-10	
3406	---	---	+ 1-10	---	---	
3303	---	---	---	---	---	
2587	---	---	+ 1-10	---	---	
3379	+ 1-10	+ 1-10	+ 1-10	+ 1-25	+ 1-25	
3318	---	+ 1-10	+ 1-10	{ + 1-10 + 1-25 }	+ 1-25	
3304	---	+ 1-10	---	+ 1-25	+ 1-10	
3080	+ 1-400					Isolated.
3335	+ 1-800					Isolated.
3320	+ 1-10	+ 1-10	---	{ + 1-10 + 1-25 }	+ 1-25	

EXPERIMENT No. 2.

Experiment to show whether a bull artificially infected with B. abortus and showing an agglutinating serum will transmit the disease to clean heifers.

This experiment was undertaken to see whether clean heifers could be infected with Contagious Abortion by running with a bull which had been artificially infected intravenously, and whose serum was showing a highly positive agglutination titre. The eight heifers used for the experiment were first tested, and, proving negative, the bull, whose agglutination was 1-800, was allowed to run with them in a large camp where no infected cattle had previously been. The bull used in the experiment had an agglutination titre of 1-10 when inoculated, and in three weeks the titre rose to 1-800, after which it gradually declined until in April, 1917, six months after inoculation, it had again reached a normal level. Tests of the heifers and bull were made at intervals for a year. Seven out of the eight heifers calved normally during the experiment, and one is still to calve, being at present close to her calving time. Three calved early in the experiment, and were therefore in calf at the commencement, but they were afterwards served by the bull, and two are again in calf, one having died. The other four calves were undoubtedly the progeny of the infected bull, and were all strong and healthy, there being no suspicion of abortion. Cow No. 3724, which calved on 31st December, 1916, died in September, 1917, and was not in calf at the time of death. The cow had always been poor in condition, and gradually got worse, finally dying of poverty. No cause for the anaemic condition could be found at post-mortem. There was no abnormality of the uterus.

Cow No.	Calved.	4.11.16.	18.12.16.	1.3.17.	29.5.17.	26.7.17.	11.12.17.
3724	30.12.16	--	\pm 1-10	—	—	+ 1-10	—
3725	19. 8.17	—	\pm 1-10	—	—	—	—
3726	9. 9.17	—	+ 1-25	+ 1-10	\pm 1-10	+ 1-10	—
3727	3. 9.17	—	+ 1-10	\pm 1-10	\pm 1-10	\pm 1-10	—
3728	4. 3.17	—	+ 1-25	—	—	\pm 1-10	—
3729	17. 3.17	+ 1-25	+ 1-50	+ 1-25	+ 1-10	+ 1-10	—
3730	—	+ 1-25	+ 1-25	+ 1-25	\pm 1-10	\pm 1-10	—
3731	20.11.17	—	+ 1-10	—	—	—	—
Bull.							
3784	—	+ 1-800	+ 1-400	+ 1-50	+ 1-25	+ 1-25	—

Conclusions.—The bull infected intravenously and showing a high agglutination titre was not a source of infection to clean cows. Since commencing this experiment an article by T. B. Hadley and H. Lothe has appeared in the *Journal of the American Veterinary Medical Association*, Vol. L, No. 2, 1916, on infection by means of the bull. They state that bulls with systemic infections of Contagious Abortion are incapable of transmitting the abortion bacillus. The evidence obtained here, therefore, supports their findings.

EXPERIMENT No. 3.

Experiment to see whether calves of infected cows showed any production of agglutinins in the serum or not.

Date.	Cow.	Agglutination of Cow's Serum at Birth of Calf.	Agglutination of Calf or Foetus.
30. 9.15	2570	+ 1-1000.....	Calf; + 1-1000.
1. 9.16	942	+ 1-1000, \pm 1-2000	Foetus full time; negative.
28. 9.16	1230	+ 1-50.....	Calf; negative.
6. 9.16	3223	+ 1-100.....	Calf; + 1-10.
4. 9.16	1148	+ 1-800, \pm 1-1000	Calf; + 1-10, \pm 1-25.
21. 9.16	3334	+ 1-500.....	Foetus at the seventh month; + 1-25.
6.10.16	3400	+ 1-25.....	Foetus; negative.
5. 1.17	2736	+ 1-200, \pm 1-400.	Calf; negative.
25. 2.17	3329	+ 1-1000.....	Calf; negative.
13. 6.17	3542	+ 1-400.....	Calf; + 1-10.
16. 8.17	3334	+ 1-500.....	Foetus; negative.
14. 8.17	3403	+ 1-1000.....	Calf; negative.
17.10.17	2664	+ 1-50.....	Calf; + 1-25, \pm 1-50.
13.11.17	3103	Not done.....	Calf; + 1-1000.
27.12.17	1148	+ 1-100, \pm 1-200	Calf; + 1-10.
24.12.17	2570	+ 1-500, \pm 1-1000	Calf; + 1-2000.

Result.—So far a positively reacting foetal serum has not been obtained, except in the case of No. 3334, where the calf was born too

small and weak to stand or suck. Two cases have occurred where the titre of the calf's and cow's serum was about the same. In the case of cow No. 2570, at two successive calvings the calf has shown a titre equal to that of the cow. In the case of calves born alive and healthy one may or may not get a weak agglutination, though in a few cases a high one is obtained, but there seems to be no definite ratio between the agglutination of the cow's serum and the calf's.

EXPERIMENT No. 4.

Examinations to find distribution of agglutinins in the different quarters of the udder.

A number of tests were undertaken with the object of studying the agglutination titre of the milk from all four quarters of an infected cow. For these milk tests milk-whey was used and was obtained from the milk by the use of rennet. The ordinary concentrated essence of rennet was used in the proportion of 1 to 80 of milk. The milk was warmed to blood-heat before the rennet was added, and a solid coagulum formed almost immediately. A sufficiently clear whey was expressed from the coagulum within an hour or two. The whey thus produced was infinitely superior to any produced by the use of lactic acid, and had the advantage of being almost clear. The titre of the milk was found to vary when taken from the different quarters of the same cow, which observation has been made by many different workers. No definite ratio could be established between the agglutination titre of the cow's serum and that of the milk. So many factors influence the titre of the milk-whey, such as the time of drawing the milk, i.e. whether at the beginning or end of milking, whether the cow is giving much or little milk, and whether the cow has just calved or is nearly dry. In a few cases where a small quantity of milk was obtained from a nearly dry udder, the agglutination titre of the milk was very high, even exceeding that of the cow's serum.

RESULT OF MILK TESTS.

Cow.	Date.	Agglutination of Cow's Serum.	Milk.				Milk from all Four Quarters.	Milk Inoculations into Guinea-pigs or Cultures made.
			Right Fore.	Left Fore.	Right Hind.	Left Hind.		
2839	19. 9. 16	+ 1-1000 ± 1-2000	+ 1-50	+ 1-25	+ 1-50	+ 1-25	—	Not done.
3201	6. 10. 16	+ 1-200 ± 1-400	Not done	Not done	Not done	Not done	+ 1-100	<i>B. abortus</i> obtained in pure culture.
3403	25. 7. 16	+ 1-4000	+ 1-500 ± 1-1000	+ 1-200 ± 1-400	+ 1-1000 ± 1-2000	+ 1-1000 ± 1-2000	+ 1-400	<i>B. abortus</i> obtained in pure culture.
3335	10. 2. 16	+ 1-800 ± 1-1000	Not done	Not done	Not done	Not done	+ 1-800	Cow almost dry.
1148	13. 9. 16	+ 1-800 ± 1-1000	+ 1-10	+ 1-10	+ 1-25	+ 1-10	+ 1-10 ± 1-25	Guinea-pig inoculation positive.
2736	5. 1. 17	+ 1-200 ± 1-400	+ 1-10 ± 1-25	+ 1-10 ± 1-25	+ 1-25	+ 1-25	Not done	Pure cultures obtained from milk.
2772	22. 6. 16	+ 1-200 ± 1-400	Not done	Not done	Not done	Not done	+ 1-800	Negative.
2889	29. 9. 15	+ 1-2000	Not done	Not done	Not done	Not done	+ 1-400	Not done.
2570	29. 6. 16	+ 1-800 ± 1-1000	Not done	Not done	Not done	Not done	+ 1-1000	Negative.
2570	10. 2. 16	+ 1-800	Not done	Not done	Not done	Not done	+ 1-800	Not done.
2227	9. 1. 17	+ 1-200 ± 1-400	Not done	Not done	Not done	Not done	+ 1-50	Guinea-pig inoculation positive.
3329	25. 2. 17	+ 1-1000	Not done	Not done	Not done	Not done	+ 1-500	Guinea-pig inoculation positive.
3542	14. 6. 17	+ 1-400	+ 1-800	+ 1-1000	+ 1-100	+ 1-1000	Not done	Pure cultures obtained from milk.
3403	25. 8. 17	+ 1-1000	+ 1-1000	+ 1-500	+ 1-1000	+ 1-500	Not done	Guinea-pig inoculation positive.

Cow 3542, which showed an infected udder on 14.6.17, aborted in 1911, since when she had calved normally three times, but the udder infection must have persisted for six years.

The milk-whey might be used as a medium through which Contagious Abortion could be diagnosed, but there does not seem to be any necessity for using it for this purpose except as a control to tests of the serum. Whatever result was obtained from an agglutination test of the milk, a test of the cow's serum would be necessary as well to control it, and a negative test of the milk would not justify a negative diagnosis. The first milk is not a very easy medium to use, as it is often thick and difficult to coagulate, and one would in any case have to bleed heifers.

In an article which appeared in the *Journal of Agricultural Research*, 7th February, 1916, Vol. 1, No. 19, Cooledge suggests the use of the agglutination test as a means of testing a cow's milk for the presence of *B. abortus*. In his experiment no tests of the cows' sera are recorded, and infection of the udder, via the teat canals, is assumed in almost every case. He inoculated 35 c.c. of a young broth culture of *B. abortus* into the right quarter of a cow whose milk had always given a negative agglutination test. The next day agglutinins had appeared in the quarter into which the culture had been inoculated, and soon after in the other three quarters almost simultaneously. Cooledge assumes that infection spread from the infected quarter to the others during milking on the hands of the milker. Though this may not be impossible, one is inclined to think that the infection from the infected quarter first caused agglutinin production in the blood, and from there the other quarters became possessed of agglutinins. The simultaneous appearance of agglutinins in the other three quarters also favours this view. No mention is made of any of the bacilli of Contagious Abortion having appeared in the other quarters. A record of another case in this article is more convincing. A cow which had never aborted was shown to have milk, which would agglutinate weakly in all the quarters except the left fore. Guinea-pig inoculations with milk from each of the three positive quarters were positive, but the left forequarter gave a negative result. Four months later the milk from the left forequarter showed agglutinins, but at the date of publication the milk had not given a positive result in guinea-pigs. The evidence here is incomplete, but is in agreement with the evidence obtained by other writers that local anti-body production to an organism can undoubtedly occur in the udder. Much importance is attached in this paper by Cooledge to infection of cows via the teat canal. Although it would be wrong to exclude this route, and it would appear to be supported by the evidence of Schroeder and Cotton that the only infected parts of a non-pregnant cow are the udder and supramammary glands, and it is known that actinomycosis can grow up the teat canal, yet it seems more likely that after the udder becomes infected originally from the blood stream, the bacilli set up a habitat there, and then infect the glands. Amongst the cows which have calved apparently normally here but showed bacilli in the milk on the day of calving, it is certain that infection did not reach the udder from the soiled hands of the milker or from discharges soiling the udder. The cows were running on a large farm, and the chances of soiling of the udder from infected material were remote.

L. H. Cooledge.—“The Agglutination Test as a means of Studying the presence of *B. abortus* in Milk,” *Journal of Agricultural Research*, Vol. 5, No. 19, 7th January, 1916.

EXPERIMENT No. 5.

The Occurrence of the Bacillus of Contagious Abortion in Milk in South Africa.

As the bacillus of Contagious Abortion had not previously been demonstrated to occur in the milk of infected cows in South Africa, attempts were made to obtain cultures from it whenever the opportunity occurred. Pure cultures were obtained from the milk of a cow which had aborted on 29th November, 1915. The cow was one of the infected herd belonging to this Institute, and had given a positive agglutination for four months previous to abortion. The foetus was never found, but must have been at about the seventh month of gestation, judging by the size of the afterbirth, which was retained, and had to be removed. The cultures were obtained directly from the milk on glucose-potato-agar and glycerinated potato. Up to this period no inoculations of guinea-pigs with infected milk had been done. In May, 1916, to see if any lesions would develop in a guinea-pig inoculated intraperitoneally with a pure culture of *B. abortus*, one was inoculated with 5 c.c. of an emulsion, about the same density as that used for the agglutination test. No lesions were found when the guinea-pig was killed three months later, but as the strain of the organism used was old, and had been constantly sub-cultured for eighteen months, another guinea-pig was inoculated with a more recently isolated strain, and died quite suddenly eight weeks after inoculation, having shown no signs of illness in the meantime. On post-mortem examination this guinea-pig showed large blood coagula in the peritoneal cavity and the spleen was enormously enlarged; length 50 mm., breadth 30 mm., and had ruptured at the posterior end. No other lesions of any organ could be found after careful search, so cultures were made from the spleen pulp and heart's blood on glucose-potato-agar. Numerous colonies of *B. abortus* developed from the spleen pulp, but the heart's blood gave no result at all. The strain isolated from the spleen grew much more vigorously than the original strain used for inoculating the guinea-pig, but was undoubtedly pure, and both culturally, morphologically, and in serological tests, it gave the same results as the original strain. Since obtaining this positive result, the milk of all cows which have aborted or calved normally while infected, has been tested by guinea-pig inoculation. In addition the stomach contents of foetuses and faeces and urine of infected cows were tested. With the exception of one case, which proved negative when the guinea-pig was killed after two months, inoculation of faeces invariably caused death, so that no definite result has been obtained from faeces of infected cows. The milk inoculations have proved very successful, positive results having been obtained in nearly every case. The only lesion so far found to be constant on post-mortem examination of our guinea-pigs after infection with abortion bacilli has been splenic enlargement in varying degrees. The spleen usually shows numerous small, white foci dotted throughout the substance, the size varying from a pin's point to a pin's head. These foci correspond to those seen in sections of infected spleens. So far we have only had two guinea-pigs which died from the infection with *B. abortus*, in both of which the splenic enlargement

was enormous; in one case the spleen weighing 24 grammes. No other macroscopic lesion was present in either of these two guinea-pigs. One of our infected guinea-pigs showed complete paralysis of the hind-quarters, but as it showed no other symptoms of disease, the paralysis was not attributed to abortion infection, though it may actually have been, as Smith and Fabyan state that they observed it in one case. Our guinea-pigs show no signs of illness as a rule, and in those killed at intervals of four to six months after inoculation recovery appears to be taking place. The serum of an inoculated guinea-pig constantly shows a high agglutination titre, rarely lower than 1-800, which is a useful fact to note when a guinea-pig is encountered which shows practically no enlargement of the spleen, and sections of the spleen do not show characteristic changes. Abortion in female inoculated guinea-pigs we have not observed, but several females have given birth to normal young, which grew at the usual rate. One such young guinea-pig was inoculated with an infected milk when it was full-grown, and when killed two months later showed the characteristic splenic enlargement. In sections of infected spleens the changes encountered were similar to those described by Smith and Fabyan, namely, foci of varying size, consisting mainly of epithelioid cells like those found in young tuberculous lesions, a few lymphoid cells, and occasional giant cells. When not in the form of foci the lesions are more diffuse, and in the much enlarged spleens large lacunae filled with red corpuscles and leucocytes usually occur. The lymphoid cells of the spleen are much increased in number.

Conclusions.—The South African strains of *B. abortus* (Bang) isolated from milk and other sources, produce typical lesions in guinea-pigs, exemplified by enlargement of the spleen in varying degrees. Other macroscopic lesions have only rarely been observed here, apart from which the disease and lesions appear to agree in every way with those observed by Schroeder and Cotton, and Smith and Fabyan in the United States of America.

EXPERIMENT NO. 6.

Experiments to find whether different lymph glands and other organs of infected animals contain the B. abortus.

These experiments were conducted with the object of confirming the statement of Schroeder and Cotton in their article, "Some Facts about Abortion Disease,"* that in the non-pregnant infected cow, the udder and its lymphatic glands, and occasionally the pelvic glands, are the only portion of the body in which abortion bacilli are to be found. Four infected cows were used, all of which were reacting to the agglutination test, and their numbers were 3334, 943, 2939, and 3547.

* E. C. Schroeder and W. E. Cotton.—"Some Facts about Abortion Disease," *Journal of Agricultural Research*, Vol. 1, No. 9, 4th April, 1917.

FOOTNOTE.—Mr. J. B. Quinlan, Lecturer in Veterinary Science at the School of Agriculture, Potchefstroom, informs me that in one of two guinea-pigs he inoculated with milk from an infected cow, he found lesions resembling those of tuberculosis in the lungs. The splenic enlargement was the only other lesion present in this guinea-pig.

Cow No. 3334.—Calved 19th September, 1916; calf alive, and at about the seventh month of pregnancy. It could not stand or suck, and was killed. The agglutination titre of the cow at the time of calving was 1-500, and she had been isolated as positive two months before. The afterbirth was retained, and the milk proved infective for guinea-pigs. The cow aborted on 16th August, 1917, the foetus being at about the fourth to fifth month, retention of the afterbirth occurred, and the milk still proved to be infected, as were also both the afterbirth and the stomach contents of the foetus. On 21st August, 1917, she was killed, and at the date of death her agglutination titre was + 1-500. Various parts were inoculated, but, unfortunately, although every care was taken, a large number of the guinea-pigs inoculated died of peritonitis, some as late as three weeks after inoculation. The cow had only two quarters to the udder, two of the quarters being congenitally absent, so milk was taken from each, and portions of each were used for inoculation. Milk was positive, portions of the right half of the udder were negative, both pelvic glands were positive, and no other positive results were obtained. The strong infection of the pelvic glands is noteworthy, the cow having aborted five days previously.

Cow No. 943.—Aborted on 30th April, 1915, before arrival here. Agglutination 1-200. Calved apparently normally on 20th September, 1916. Agglutination of cow at birth of calf was 1-800. The afterbirth was not retained, but the cow suffered from metritis for a month after calving. The milk was not tested. Killed on 29th August, 1917, on account of a dislocated hip, and on post-mortem a normal foetus was found in utero, with foetal membranes normal in appearance. Agglutination of cow at date of death was 1-100 to 1-200, foetus negative. The cow was dry when killed, so no milk was inoculated. Of the guinea-pigs inoculated from this cow, one surviving from inoculation with the left forequarter of the udder was negative; both hind-quarters were positive, although one guinea-pig from the left hind was negative, the other being positive. Both supramammary glands were positive, the pelvic glands negative, as were fluids from the thoracic duct and bile. Guinea-pigs inoculated with uterine material and stomach contents of the foetus died of peritonitis.

Cow No. 2939.—Aborted 4th July, 1915; afterbirth retained, and pure cultures of *B. abortus* were obtained from it. The agglutination titre of the cow at the date of abortion was 1-2000. The cow aborted again on 25th August, 1916, the calf this time being fully developed, but born dead. The milk, afterbirth, and stomach contents of foetus were all infected, as shown by guinea-pig inoculation. A third abortion occurred on 20th June, 1917. The foetus was again fully developed, and the afterbirth retained, and on inoculation into guinea-pigs, the afterbirth, stomach contents, and milk were again positive. On 6th September, 1917, an attempt was made to remove the udder of the cow, but although the operation was successful the animal did not survive it for more than a day, death being undoubtedly due to delayed chloroform poisoning. Various portions of the cow were used for guinea-pig inoculation; the udder, milk, supramammary glands and gastric glands proved positive, though the result in the case of the gastric glands may probably be due to the cow having eaten a portion

of her infected afterbirth at the abortion three months previously. Portions from all four quarters of the udder were mixed, and not inoculated separately.

Cow No. 3547.—Aborted in 1910. When first tested in 1914, her agglutination titre was 1-1000. This cow never calved again during the seven years after she aborted, and at the time of death, which was due to traumatic pneumonia, the agglutination was only 1-50. The uterus at post-mortem was not pregnant, and contained a quantity of yellowish brown mucus. The results of guinea-pig inoculation from this cow are as follows:—The udder, supramammary glands, and pelvic glands were positive; all other parts negative.

Discussion.—The results obtained here correspond with those of Schroeder and Cotton, with the exception that one guinea-pig inoculated from gastric glands proved positive. With the exception of two guinea-pigs inoculated from the udder and supramammary glands of Cow No. 3547, and those from milk, afterbirth, and stomach contents, none of the inoculated guinea-pigs has shown any definite lesions, and their agglutination titres have been taken as the indicators of infection. There seems little doubt that the udder and its lymphatic glands are the only infected parts of a non-pregnant infected cow, but how the udder is infected originally is not settled. Allowing for a small proportion of cases where infection originally occurred, via the teat canals, and this proportion must of necessity be small, particularly under ranching conditions, one is left with two possible channels by which the bacillus may reach the udder. The one channel is via the blood stream direct to the udder after absorption from the alimentary tract or from the vaginal mucous membrane in infection via that channel. The other is by the pelvic glands to the supramammary glands, and thence to the udder from an infected uterus. Schroeder and Cotton have proved that by infecting the udder the uterus can become infected, but the reverse process probably occurs as often. Infection can apparently travel along lymph channels in the opposite direction to the lymph stream, and this undoubtedly occurs in tuberculosis of the udder. MacFadyean considers that tuberculosis of the udder is usually the result of infection via the lymph stream from lesions in the abdominal cavity. It is not known whether it is necessary for the udder to become infected before the uterus can, whatever the point of entry of infection, but further evidence on this point may be forthcoming.

Material Used.	Cow No. 3334. Agglut. (21.8.17) = + 1-500.	Cow No. 943. Agglut. (29.8.17) = + 1-200.	Cow No. 2939. Agglut. (7.9.17) = + 1-1000.	Cow No. 3547. Agglut. (6.11.17) = + 1-50.
Milk.....	(1) Lesions, agglut. = + 1-2000 (1) " " "	Cow dry	(1) + 1-400 ; } Both showed (1) + 1-1000 ; } lesions	Dry.
Left forequarter.....	{ Died	(1) Negative agglut. } (1) Died } No lesions		
Left hindquarter.....		Died		
Right forequarter.....		Died		
Right hindquarter.....	{ One lived ; showed no lesions	(1) + 1-100 } (1) + 1-200 } No lesions		(1) + 1-50 ; no lesions (1) + 1-1000 ; showed lesions.
Right suprarenal gland....	Died	(1) Died (1) + 1-50 ; no lesions	(1) + 1-50 ; no lesions	{ (1) + 1-400 ; showed lesions.
Left suprarenal gland.....	Died	(1) + 1-200 ; no lesions	(1) + 1-100 ; no lesions	
Pelvic glands.....	(1) Agglut. = + 1-1000 } No (1) Agglut. = + 1-2000 } lesions	(1) No agglut. } (1) " } No lesions	(1) No lesions } + 1-10 (1) " } (1) " }	(1) + 1-50 ; no lesions.
Thoracic duct.....	Not done	Negative	Not done	Not done
Mucus from uterus.....	Died	Died	(1) + 1-10 ; } (1) " } no lesions	(1) = } No lesions. (1) = }
Mesenteric glands.....	Died	Not done	(1) = } No lesions (1) = }	(1) No agglut. ; } no lesions. (1) " } (1) " }
Gastric glands.....	Died	Not done	(1) + 1-25 ; } No lesions (1) + 1-100 ; }	(1) Negative ; } no lesions. (1) " } (1) " }
Tonsils.....	Died	Died	Not done	Not done
Stomach contents of foetus....	(1) Agglut. = + 1-2000 } Showed (1) " } lesions	Died	(1) + 1-2000 ; showed lesions	Not done
Afterbirth.....	(1) Agglut. } Showed lesions (1) " }	Died	Not done	Not done
Bile.....		(1) Negative ; no lesions	Not done	Not done

EXPERIMENT No. 7.

The Lesions produced in Guinea-pigs by the B. abortus.

Attention was first drawn to the lesions produced in guinea-pigs after inoculation with the *B. abortus*, by Theobald Smith and Marshal Fabyan. In their article on "The Pathogenic Role of *B. Abortus* (Bang)" in the *Centralblatt für Bakteriologie*, Abt. 1, Vol. 61, No. 7, Jena 1912, a full description of the lesions produced in guinea-pigs is given. The discovery of the pathogenic effect in guinea-pigs was made during the inoculation of necrotic cotyledons of a cow's after-birth, tuberculosis having to be excluded. Smith and Fabyan in their first case observed great enlargement of the spleen which was engorged with blood. The lymph nodes of the subcutaneous tissues were enlarged, the liver showed here and there small depressions and was studded with small yellow nodules. Clinical lesions described were stiffness of different joints, occasional affection of an eye, paralysis of both hindquarters, also rupture of the spleen was observed, but these were not constant and could only be regarded as of occasional occurrence. The general description of the lesions found was that in all a more or less distinct polyadenitis was present, the spleen was enlarged to as much as eight times its normal size, and the liver, though not as a rule much changed in colour or consistence, showed its surface to be pitted with small scars. A rare condition was the occurrence of small yellow nodules in the liver, and the kidneys were sometimes normal, sometimes very much affected, being enlarged, whitish, and the outer layer dotted with small grey areas. In males the testicles were nearly always atrophied or unevenly enlarged. The lungs were often studded with small grey nodules resembling tubercles. A few animals showed bone affections taking the form of deformation of one or more whole ribs, with corresponding enlargement of the narrow cavity. In one case the spinal column was affected and in others the bones of the foot. The authors found that death was an uncommon sequel to infection, and in one case a guinea-pig which was allowed to live for eleven months was in good health at the end of that time.

Schroeder and Cotton in an article on "The Bacillus of Infectious Abortion found in Milk" in the Twenty-eight Annual Report of the Bureau of Animal Industry of the United States, 1911, describe similar lesions and give photographs contrasting the macroscopical appearances of the lesions of Contagious Abortion and Tuberculosis in guinea-pigs. Briefly their description is as follows: No well-marked lesions are developed until after the passage of six weeks or more. Extreme enlargement and oedema of the lymph glands is present. Small glistening nodules occur in the lungs, later forming large necrotic areas. The spleen is often 30 to 40 times the normal size and shows small white spots up to several centimetres in diameter. Enlargement and degenerative changes take place in the liver, which is thickly beset with pale yellow and dirty white areas due to an enormous proliferation of connective tissue. A diffuse parenchymatous nephritis occurs, which reaches stages when dense fibrous nodules are found in the cortex of the kidney. Degeneration of the testicles, resulting in the conversion of one or both of them, into structureless cysts containing creamy pus, may occur. These lesions are given as those most frequently met with, any combination of them

being likely to occur, but the splenic lesion is always present. It will be seen that the descriptions of the disease as it occurs in the United States tally fairly well. Schroeder and Cotton worked with milk, and Smith and Fabyan with cotyledons or other infected material, except milk.

T. C. Evans, in the Report of the Director-General for the Canadian Veterinary Service, 1914, describes the lesions occurring after the inoculation of guinea-pigs with infected milk in Canada. His description agrees with that of Schroeder and Cotton, with the reservation that the lesions he found were less pronounced, the difference according to him being due to his guinea-pigs having less resistance on account of the cold quarters they were kept in, or that the Canadian strains were more virulent. Most of his guinea-pigs died from the infection, which must have been virulent, as most workers have found that death is not a usual sequel.

Since commencing the study of the lesions produced by the inoculation of infected milk and other materials in South Africa, the writer has made post-mortems on about 200 guinea-pigs inoculated with every variety of infected material and killed at different times after inoculation, varying from four weeks to seven months. Enlargement of the spleen which has always been taken as indicative of a successful infection, has occurred in all the positive cases, but no other lesion has occurred with any constancy; in fact, until about thirty post-mortems had been made no other macroscopic lesion was met with at all. Very careful post-mortems have been made of all the guinea-pigs killed, and so far, only three cases have been met with where any changes in the liver were observed. All three were from cases where milk had been used for inoculation. In one, where the spleen was not markedly enlarged, 30 mm. by 15 mm., the liver showed numerous grey pin-point foci throughout, which could only be seen properly with a hand lens. In both the remaining two the foci in the liver were very numerous, yellow in colour, and varying in size from being just visible to that of a pin's head. One of the latter two cases occurred in a guinea-pig inoculated with milk from one quarter of an udder, the remaining quarters giving splenic enlargement only. Lymphadenitis has not been met with; at least not of a macroscopic nature. The only case met with where any gland lesions were present was that of a guinea-pig which had a small abscess at the site of inoculation on the abdomen. In this animal all the mesenteric glands showed great enlargement and numerous centres containing greenish yellow pus were seen on incising these glands. Peritoneal adhesions were present and the spleen and liver showed a few of these purulent foci, but not other organs were involved. Pure cultures of the *B. abortus* were obtained from the pus in these foci, both splenic and in the mesenteric glands. This case must be regarded as exceptional, as in no other guinea-pig has any enlargement of a lymphatic gland been seen. One case occurred of paralysis of the hindquarters and one in guinea-pig that carried its head in a peculiar manner turned to one side. The head of the latter animal was carefully examined at post-mortem for any sign of parasitic infection of the ears with negative results, and no changes were observed in the internal ear, or brain. In both these cases the symptoms may have had no connection with the *B. abortus*, but both guinea-pigs were undoubtedly infected. No changes have been noted in any of the bones, such as the ribs, a most careful search having been made for macroscopic alterations. The

typical spleen as encountered in the majority of cases was about 30 to 40 mm. in length by 20 to 25 mm. in breadth and weighing 8 to 10 grm. The appearance of the surface under the capsule was usually uneven and lumpy, and speckled all over with greyish-white foci, varying considerably in size, though rarely larger than 2 to 3 mm. in diameter. A large number of dark reddish-brown foci of similar size were usually present, as well and probably correspond to the lacunae filled with blood, seen in sections of spleens. In only one case were large white areas found in the spleen. These areas, of which there were two, were about 8 mm. by 4 mm., and both were on the posterior border of the spleen and extended through the thickness of it. Two cases have occurred here of enormous enlargement of the spleen. One guinea-pig which died two months after inoculation with a pure culture of the *B. abortus*, showed rupture of the spleen, which was so large as almost to fill the abdominal cavity. The length was 70 mm. and width 35 mm. The pulp was soft and broke down very easily. In the other case the spleen was 70 by 30 mm., weighed 24 grm., and on section the pulp was reddish-black in colour and exuded on cutting the organ. It cannot be said that any difference occurs in the severity of the lesions produced by inoculation of infected material from different sources. The most severe cases met with here have been inoculated with milk, but those inoculated with material such as cotyledons from infected afterbirths and stomach contents of aborted foetuses, perhaps show pronounced lesions with more regularity, probably on account of such materials being heavily infected as a rule. In the great majority of cases, the agglutination test has been applied to the sera of the infected guinea-pigs and has been found of very great use, particularly when the post-mortem lesions were not well developed. It has been found that the sera of practically all guinea-pigs showing good post-mortem lesions will give a water-clear agglutination in a dilution of 1-1000 or often 1-2000 and higher. The chief usefulness, however, is where the spleen is only slightly, if at all, enlarged, and not abnormal in appearance. Such cases have occurred here, and the agglutination test has often in such cases given a positive result. Definite agglutination in a dilution of 1-50 or over has been taken as positive, though agglutination, even in a 1-10 dilution, may be positive, as normal guinea-pigs do not seem to possess normal agglutinins for *B. abortus*. One may find a spleen only slightly enlarged, say 30 mm. by 15 mm., and still the serum of the guinea-pig will give a positive agglutination in as high a dilution as 1-1000. Even histological examination of a spleen which appears only slightly enlarged will not always show any of the typical changes which occur in this disease in guinea-pigs, so that the best method to adopt is to combine macroscopical appearances, histological examination and the agglutination test, when deciding as to whether a guinea-pig is infected or not.

HISTOLOGICAL EXAMINATIONS.

Spleen.—In sections of the spleen stained with haemalum and eosin, one can, in a good case, see even with the naked eye, numerous small pale staining areas one to two millimetres in width, irregular in shape, though as a whole fairly circumscribed. These foci correspond to those seen on microscopical examination. To the naked eye lacunae containing blood are visible in sections and are seen to be of

greatly varying size, though usually they are small and correspond roughly in size with the epithelioid cell foci, which are the pale-staining areas previously mentioned. In sections of normal guinea-pig spleens one can see the malpighian bodies quite distinctly, and in a *B. abortus* infection one sees that they may be occupied by the pale-staining foci, though the latter may occur anywhere in the spleen-pulp. Normally a malpighian body of the guinea-pig's spleen shows a pale area in the region of the bloodvessel supplying it, but in abortion infection it may show only a thin rim of lymphoid cells surrounding a focus of epithelioid cells.

Microscopical Examination.—The most striking feature of the disease caused in guinea-pigs by the *B. abortus* is the presence of foci of varying size composed chiefly of epithelioid cells. These large pale-staining cells resemble those found in tuberculous lesions and in glanders nodules. The foci are sometimes well defined, sometimes quite irregular in outline and appearing to infiltrate the whole spleen. In a typical case the foci are composed mainly of epithelioid cells, a few polymorphonuclear leucocytes being present and some of the original lymphoid cells of the spleen. Where extensive changes have occurred and the whole spleen is infiltrated with epithelioid cells, there may be no traces left of malpighian bodies. Giant cells are frequent, often very numerous. Lacunae are constantly present appearing as areas, irregular in shape, crammed with red blood corpuscles and leucocytes. As a rule the blood corpuscles are intact, but in some cases a lacuna with blood in it may be converted into a coagulum which may even be undergoing resolution. Necrosis in the spleen has not been met with, even in good cases. The epithelioid foci appear not to undergo necrosis with the advance of the disease, and would appear to undergo resolution after about three to four months, as in cases left for six months or longer, the spleen may not show any changes at all on histological examination, although the blood serum is still strongly positive to the agglutination test. In each of the two cases which died from the disease the spleen was practically converted into a blood coagulum. On section in both cases, traces of the original splenic tissue were still present, but the whole organ was infiltrated with blood, coagulated in some parts, but for the most part not. In these cases foci of epithelioid cells were no longer visible, and must have undergone necrosis, rupturing the blood-vessels of the spleen and causing the tremendous infiltration with blood seen in sections. The enlargement of the spleen constantly seen in infected guinea-pigs must be due to a hypertrophy of the normal splenic tissue, as neither the lacunae nor the foci could account for it in most cases.

Liver.—To the naked eye, sections of an infected liver sometimes show visible foci about the size of a pin's head or smaller. The majority of good cases of abortion infection in guinea-pigs show lesions in the liver of a microscopical nature, although to the naked eye there may be no apparent changes. In the case of the section of liver shown in photo 1, the focus there shown was distinctly visible without magnification as were others even smaller in size in other parts of the section.

Microscopical Examination.—In the liver, foci of epithelioid cells occur similar to those found in the spleen, but vary in structure. Some appear as irregular clumps of epithelioid cells mixed with

lymphoid cells in varying proportions according to the age of the lesion, and including a few polymorphonuclear leucocytes. The more advanced lesions are composed almost solely of epithelioid cells. Giant cells occur, but are much rarer than in the spleen. The foci in the lobules themselves are usually well defined and composed of two zones, a central and an outer. The central one consists of hepatic cells distorted in shape, but not broken down, and a large number of polymorphonuclear leucocytes, many of which have undergone karyorrhexis. The distorted hepatic cells when stained with Sudan 3 do not show any signs of fatty degeneration. The outer zone is composed entirely of epithelioid cells. According as to whether one's section has cut the centre of a focus or not, one may find the central zone large, small, or absent. This type of focus shows a considerable amount of resemblance to a glanders nodule of the guinea-pig's spleen, though it does not show advanced necrosis in the centre, even in old cases. Livers of infected guinea-pigs show very little change of a fatty nature, sections showing only a few odd liver cells to have undergone fatty changes. *Kidneys*: So far, even microscopically, no changes have been found in the kidneys. *Lungs*: In sections of lungs from infected guinea-pigs areas are to be seen where great proliferation of the endothelial cells has taken place. The alveoli in these portions have practically disappeared. It was thought that perhaps in a normal guinea-pig's lungs the interalveolar tissue might be better marked than in other animals, but sections from the normal lung of a guinea-pig do not show this to be the case. No giant cells were present and no necrosis was seen in any of the lungs examined. *Testicles*: No lesions were seen.

Examination for B. abortus: A number of different stains have been tried in the attempt to stain the abortion bacilli in sections of infected organs. So far none of the attempts have been successful, but further work may produce successful results. The stains tried have been Thionin alone, MacFadyean's method for glanders bacilli in sections, Saathoff's methyl-green, pyronin method, Gram Weigert after fixing in Tellyesniezky's fluid with the addition of formalin (a method which causes gram negative organisms to retain the gram stain), and finally the Giemsa (wet) method. Levaditi's method for staining spirochaeta pallida in sections was tried without success. The fact that the organism is so minute, and decolorized by Gram's method, renders it difficult to stain it, and if the clumps of bacilli are intracellular they are difficult to distinguish from cell granules. In smears made from very young growths of *B. abortus* on agar which have been made from infected spleen pulp, one can see that clumps of the bacillus are present in some of the leucocytes appearing to have burst out of the cells by their growth.

CONCLUSIONS.

The disease produced in guinea-pigs by infection with the *B. abortus* belongs to the class of the so-called granulation tumour diseases, which includes tuberculosis, and is noteworthy on account of its extremely chronic nature, low mortality, and tendency to recovery as compared with tuberculosis and glanders in this animal, to which diseases it shows many points of similarity. As compared with the disease experimentally produced in guinea-pigs in America,

the South African form would appear to be even more benign than that described by Theobald Smith and Marshal Fabyan. As has been mentioned in this paper, in some cases the splenic enlargement has been so small that only histological and serological methods have proved them to be positively infected.

FIG. 1.

Section of a focus in the liver of an infected guinea-pig, showing a central zone composed of liver cells distorted and swollen, but still retaining the stain and numerous polymorphonuclear leucocytes, which show karyorrhexis surrounded by a zone composed entirely of epithelioid cells. The karyorrhexis in the central zone can be distinctly seen.

FIG. 2.

Section of a focus in the spleen of an infected guinea-pig. The diffuse nature of the changes shows up well in the photograph, the mass of epithelioid cells not being so well defined as in the liver, and portions of the splenic tissue are still visible. A giant cell is present in the lower half of the section, about the centre, and in the spleen from which the section was made, giant cells were particularly frequent. No necrosis is present.

EXPERIMENT No. 8.

The Effect produced by the B. abortus (Bang), on the X-rayed Guinea-pig.

As the *B. abortus* appeared from our own observations and those of other workers to produce changes in the spleen of a pathological nature, though it has not been observed here as yet that any other macroscopical lesions occur except the splenic and occasionally the hepatic, the method of reducing the lymphoid tissue of the body by exposure to X-rays was tried in order to see if the lesions could be produced in a shorter time in guinea-pigs thus exposed. The usual time necessary for the productions of lesions in a guinea-pig is about two to three months, so any method which reduced this period would be of value. These experiments were suggested by an article which was published in the *Journal of Experimental Medicine* for October, 1916, on "A rapid method for the Diagnosis of Renal Tuberculosis by the use of the X-rayed Guinea-pig," by J. J. Morton, M.D. He produced lesions of tuberculosis in a week to ten days in X-rayed guinea-pigs, which had been inoculated with known tuberculous urine, the usual time required for the production of lesions being five weeks. This paper is exceedingly interesting, and is based on the assumption that the lymphoid tissue of the body constitutes an important agent in the defensive mechanism against tuberculosis. The results produced in his cases were very striking, caseous nodules of various sizes being found in the spleen, and caseous lymph glands, etc., after ten days, the lesions varying according to the severity of the infection. Only one exposure to X-rays was given for the sake of convenience, though the author thought that a series of small exposures, if possible, would have been better. Each guinea-pig was radiated for ten minutes with a Coolidge tube, being placed in a cardboard box, so that it could not

move round. The target was twelve inches from the base of the box. A five milliamperere current was passed through the tube backing up eight inches of spark between the points, the machine employed being the Snook Interrupterless, and no filters were used.

The method as described by Morton was followed here in exact detail, the X-ray apparatus at the Pretoria Hospital being used by the permission of the Resident Medical Officer. As the dose given by Morton was not stated, the dose given to guinea-pigs here was carefully measured, and found to be 40 x.

Three guinea-pigs were exposed to X-rays on 24th December, 1916, for ten minutes each, but owing to a mistake having been made in the distance of the target from the base of the box a dose of only 20 x was given to each animal. Three days later, on 27th December, 1916, these guinea-pigs were inoculated as follows:—(a) and (b) were given 2 c.c. of a pure culture intraperitoneally, the strain being (1148), one which was known to cause lesions in guinea-pigs, and the culture was given in the form of an emulsion from an agar slope in 2 c.c. or normal saline, the thickness being about that used in the agglutination test, (c) was given 5 c.c. of milk from a cow which had been showing abortion bacilli in the milk two months previously. No other naturally infected material was at hand at the time. The milk was given intraperitoneally, and a control normal guinea-pig was inoculated at the same time.

(a) Was killed on 10th January, 1917, and on post-mortem no lesions of any kind were found. Cultures from the spleen pulp on potato agar gave numerous colonies of *B. abortus* after two days' inoculation, but no cultures were obtained from the blood and peritoneal fluid. On the day of death this guinea-pig's serum gave a water clear agglutination in a dilution of 1-1000, and the leucocyte count was 4900 per cmm. This agglutination is rather interesting, since some authors have suspected the lymphatic system of being the seat of production of agglutinins. This is not proof to the contrary, but is evidence that the agglutinin production is not affected by X-rays. (b) Was allowed to live in order to see whether it would die later. It died in March, 1917, had a large spleen, and pure cultures were obtained. (c) Was killed on 12th January, 1917, and on post-mortem no lesions were found. Cultures from the spleen, blood, and peritoneal fluid all gave negative results. A control to this guinea-pig was killed the same day, and gave negative results as well.

No conclusion can be arrived at from this first experiment, which was only a provisional one, and good results were not anticipated, as the dose of X-rays given was too small. The positive result in the case of (a) could not be regarded as of much importance, as a control had not been used, but it showed that cultures might be obtained from a spleen which showed no macroscopic alteration. The negative result in the case of (c) might possibly have been due to the absence of *B. abortus*, as the milk, although infected two months before, might have become free again in the meantime.

On 21st January, 1917, three more guinea-pigs (d, e, and f) were exposed to X-rays for ten minutes each, the dose this time being 40 x. The guinea-pigs stood the operation well, and no depilation occurred afterwards. On 24th January, 1917, they were inoculated as follows:—(d) Was given 2 c.c. of a thin emulsion of a pure culture (942) which was known to cause lesions in guinea-pigs. The emulsion

was about the thickness of agglutination test fluid, and was made in normal saline, the dose being given intraperitoneally. A control guinea-pig was inoculated at the same time and with the same dose.

(e) Was given 2 c.c. intraperitoneally of a culture (2939) which had previously caused lesions in guinea-pigs. The emulsion was similar to that given to (d).

(f) Received 5 c.c. of milk intraperitoneally from a cow, No. 2736, which had just calved after a previous abortion, and was showing *B. abortus* in the milk. This milk was the only available material infected naturally. A control normal guinea-pig was inoculated with the same milk.

Guinea-pig (e) died in the night of 31st January, 1917, and on the following morning was already too decomposed for any cultural work to be done with it. The previous day it appeared quite healthy, or otherwise it would have been killed, as during the summer months, October to March, a few hours may be sufficient for putrefaction to reach an advanced stage.

(d) Died on 31st January, 1917, and on post-mortem showed no lesions of the *B. abortus* type, and death was put down to peritonitis, as there appeared to be inflammation of the abdominal organs. Cultures were made from the heart's blood, and gave positive results after five days' inoculation. Only the heart's blood was used for cultural work, as the animal was somewhat decomposed, having died in the night. This result was somewhat unexpected, and immediately the cultures proved positive the control guinea-pig was killed on 6th February, 1917. It appeared perfectly well when killed, and on post-mortem showed no lesions of any kind, but cultures were obtained from the spleen pulp, peritoneal fluid, and heart's blood, without difficulty. It would appear that *B. abortus* may be present in large numbers in the spleen without causing any macroscopic change, as was also found in the case of guinea-pig (a). It is possible that the guinea-pig (d) died from a *B. abortus* septicaemia, though its control might have shown the presence of the organism if it had been killed when (d) died. The X-rayed guinea-pig (f) inoculated with milk on the 24th January, 1917, was killed on 8th February, 1917. Post-mortem: No lesions of any kind were observed, and cultures from the blood, spleen, and peritoneal fluid failed to give any growth of *B. abortus*. The control normal guinea-pig to this one proved negative, both at post-mortem and in cultures from its spleen, blood, peritoneal fluid, etc.

As the results obtained from the previous guinea-pigs were not completely satisfactory, two further guinea-pigs were X-rayed on 17th July, 1917. As the previous three were somewhat undersized, and weighing 400 grm. each, two large ones each weighing 600 grm. were used. These two guinea-pigs (g) and (h) each received a dose of 40 ×. On 19th August, 1917, they were inoculated as follows. Each animal was given 5 c.c. of milk from a cow, No. 2706, intraperitoneally. This milk contained no abortion bacilli, but to it were added virulent abortion bacilli from a culture, about half a small platinum loop full of surface growth being mixed with 20 c.c. of milk. The material on the loop did not come off in the milk altogether, so that the infection was not so heavy as would appear. Infected material was again not available as the X-raying could not be managed at the time such material was on hand. Milk from cows which had been excreting bacilli in the milk at some previous time was available, but the possibility of such milk being non-infected at the time when the X-rayed

guinea-pigs were to be inoculated, and the small number of guinea-pigs available, prohibited its use. These two guinea-pigs remained in good health until they were killed. A control normal guinea-pig was inoculated at the same time with the same material.

Guinea-pig (*g*) was killed on 2nd August, 1917, fourteen days after inoculation. No lesions were found on post-mortem. Spleen pulp was inoculated on to slopes of Stockman's glucose potato agar and colonies of *B. abortus* appeared after three days' inoculation. The agglutination titre of this guinea-pig's serum at the date of death was $1 \cdot 10 \pm 1 \cdot 25$. The control guinea-pig was killed on 3rd August, 1917, and showed no lesions at all on post-mortem. Cultures were made from the spleen pulp, blood, and peritoneal fluid, of which the spleen pulp only gave colonies of *B. abortus* after three days' inoculation, and these colonies grew at a quicker rate than those from the X-rayed one, the medium being exactly the same and from the same batch. The agglutination titre of the control guinea-pig's serum at the date of death was 1-10, so that there is practically no difference between it and the X-rayed one. The guinea-pig (*h*) was killed on 3rd September, 1917, and the only lesion found on post-mortem was enlargement of the spleen, length 32 mm., width 12 mm. The spleen had the uneven surface usually found in cases of infection with *B. abortus*, and probably due to enlarged lymph follicles. From this last experiment it will be seen that there is no apparent difference in the severity of a *B. abortus* infection in an X-rayed guinea-pig and a normal one.

The guinea-pig (*g*) showed an agglutination titre of only 1-10 after fourteen days, while the guinea-pig (*a*), which only received a dose of X-rays of 20 x, had a titre of 1-1000 at the time of death. It is probable that the larger dose of X-rays had the effect of lessening the agglutinin production, as the guinea-pig (*h*), which was allowed to live for six weeks, had a titre of 1-500 at the time it was killed. Most infected guinea-pigs have a titre of at least 1-1000 six weeks to two months after inoculation. It has been observed that large doses of X-rays given after inoculation with blood corpuscles may restrain and delay lysin production, so that in the guinea-pigs used in the last experiment (*g*) and (*h*) a similar effect was produced.

Microscopical Examinations of the Organs of the X-rayed and Control Guinea-pigs.

In only two of the X-rayed guinea-pigs or controls were any lesions found which could be attributed to infection with the *B. abortus*. In one of these (*h*), which was not killed until six weeks after inoculation, small foci containing epithelioid cells were found in sections of the spleen, but the other organs were normal. The other one (*b*), which died ten weeks after inoculation, great splenic enlargement of a typical nature was found, but a histological examination was not made as the organs were not kept, the animal's death having occurred during the writer's absence. Leucocyte counts were done on the blood of one X-rayed guinea-pig (*d*) at intervals of two days. On the day before being X-rayed, the leucocyte count of the guinea-pig was 8000 per cm., and the gradual lowering of the count until the death of the animal on the eighth day after X-raying can be seen from the attached chart. The number of lymphocytes had, two days before death, been reduced from the average normal somewhere in the region of 40 per cent. to 15 per cent. The guinea-pig was a small one, weighing about 350 grm., but was in good health and fat before being X-rayed.

One is forced to conclude from the experiments undertaken in this paper, that reduction of the lymphoid tissue of the guinea-pig's body does not render the animal more susceptible to infection with the *B. abortus*. The disease closely resembles in many ways that produced by the tubercle bacillus, particularly in the length of time taken to produce lesions, and the work of many investigators on the occurrence of the tubercle bacillus in the milk of cows leaves little room for doubt that a considerable portion of the guinea-pigs examined post-mortem showed a pure infection due to *B. abortus*, or tuberculosis and abortion infection in association. Schroeder and Cotton freely admit this, and Smith and Fabyan mention the so-called pseudo tuberculosis which many investigators met with when testing suspected tuberculous milk. Except for the short time required by *B. mallei* to produce lesions and death in the guinea-pigs, there is a close resemblance between Glanders and Abortion disease as regards the lesions produced in the animal. Histologically, however, the lesions produced in the spleen are not similar. Glanders infection produces definite nodules, such as are found in the lungs of the horse, which nodules are well marked off from the normal tissue of the spleen, and consist of a central zone undergoing necrosis, and showing distinct chromatotexis in the cells of the part, surrounded by a zone of epithelioid cells. In abortion infection in guinea-pigs one may get sharply marked off foci of epithelioid cells in the spleen, but it is very unusual to find necrosis in the centre, and the disease assumes a more or less diffuse character, the epithelioid cells not being necessarily in definite foci at all, but infiltrating the whole of the spleen tissue. In the liver one may find foci resembling Glanders' nodules, histologically with the difference that the central portion does not show necrosis, although the liver cells may be distorted in appearance. In a recent paper published by M. J. Sittenfield in the *Journal of Cancer Research*, and entitled "The Significance of the Lymphocyte in Immunity to Cancer," the author contradicts the statements of various workers to the effect that an increase of mononuclear lymphocytes is associated with immunity to implanted tumour grafts. Rous, Murphy, and Morton attempted to experimentally prove that a depletion of the lymphoid elements produced by X-rays in repeated doses was associated with loss of resistance, natural or induced, to tumour inoculation. They emphasized the importance of this lymphoid reaction in resistance to experimental tuberculosis as well. Kessel and Sittenfield contradicted this and showed that in experimental tuberculosis X-raying caused healing by fibrosis, so that life was actually prolonged. In the paper on the "Significance of the Lymphocyte in Cancer Immunity," two methods of producing hyperlymphocytosis in white rats were used (1) by means of subcutaneous inoculation of pilocarpine, (2) intravenous inoculation of leucocyte cream obtained from X-rayed rats rich in lymphocytes after repeated small doses. In a third experiment immune rats, whose absolute immunity had been tested by repeated tumour inoculation, were subjected to large and repeated doses of X-rays, producing a marked reduction in lymphocytes. On inoculation of all three lots with tumours no increase or decrease could be noted in their resistance to tumour inoculation. These results, and those of Morton in the *Journal of Experimental Medicine*, and which formed the suggestion for the present paper, are contradictory, but much work is being done at present in regard to the rôle of the lymphocyte in immunity, and

fresh evidence may be expected, particularly from the host of workers now engaged on the cancer problem and allied ones.

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IX.—CULTURAL WORK.

Very little has been done in trying various media for the growing of *B. abortus*. The medium at present in use here for the growing of *B. abortus* for emulsions for agglutination tests, etc., is glucose-glycerine potato agar, made according to the method described by Stockman in the *Journal of Comparative Pathology and Therapeutics* for September, 1914. This medium has given excellent results, strong growths being obtained without difficulty on it. Even for primary cultures from infected material it has proved very useful, and growths on it were nearly always obtained in two or three days from milk when it was sufficiently infected to give growths in primary culture. Having noticed that in the case of Jones' disease, the medium for the growth of the organism was greatly improved by the addition of dead tubercle bacilli or other acid fasts, it was thought that perhaps the addition of a strong emulsion of killed abortion bacilli to a medium on which the organism did not grow vigorously, might improve it for the organism. Ordinary agar on which *B. abortus* grows moderately well was used, and to it was added a thick killed emulsion of *B. abortus* in the proportion of 1 of emulsion to 10 agar. This medium was inoculated with a vigorous strain of *B. abortus* and controls were used of the agar without the emulsion of bacilli. No difference was noted between the rate of growth of the culture inoculated on the emulsion agar and that on the control, the growths in both cases being very moderate and not nearly as luxuriant as on the Stockman medium.

A medium made by adding 2 per cent. agar to a thick emulsion of *B. abortus* in .85 per cent. saline solution was a complete failure, no growth being obtained on it at all, probably owing to there being insufficient nitrogenous material in the medium.

In two cases, Cow No. 942, which had just aborted, and a calf, No. 3771, which was just newly born in an apparently normal manner to an infected cow, attempts were made to isolate the bacillus of abortion from the blood by Gildermeister's method. This consists in adding one part of fresh blood to nine parts of boiled distilled water and incubating for twenty-four hours, after which plates are made, and again after forty-eight hours' incubation. No result was obtained, which agrees with the findings of Schroeder and Cotton with blood inoculated into guinea-pigs.

X.

A table of the histories of the infected cows which have aborted in the Contagious Abortion experiments since the commencement of investigations into the disease in 1913.

As will be seen from the following table (I), two cows have aborted three times, four twice, and seventeen once only; 28 other cows did not abort at all, calving normally, remaining non-pregnant, or dying. Of these 28 cows, 24 calved normally, some several times in succession, and in several cases infection of the milk and after-birth with abortion bacilli was proved, although the calvings were, to all appearances, normal. Those cows which aborted and have not calved normally since have been kept isolated by themselves in a herd (A), which therefore includes Cows Nos. 942, 3403, 3040, 927, 2737, 3335, and a bull. Cow No. 942 was included because, although she had a live calf at the last calving, her afterbirth was retained and strongly infected with abortion bacilli. All the other infected cows are included in a herd (B).

Table II contains a list of the cows which have never aborted although infected, with the number of calvings. In this table, as in Table I, infected means having an agglutination titre of 1-100 or over.

Of those which died in Table II, in no case could death be put down to any sequel of Contagious Abortion, most of them dying of Heartwater, or as a result of exposure to bad weather when they were in poor condition.

TABLE I.

(Cow.	Date of First Abortion and Age of Foetus, where known.	Date of Second Abortion and Age of Foetus, where known.	Date of Third Abortion and Age of Foetus, where known.	Subsequent History.
942	2. 7.13 — 4th month	15. 6.15 — 5th month	28. 8.16 — 9th month	Calved 21.10.17; calf strong and healthy; cow's afterbirth retained; isolated in herd (A).
2939	4. 7.15	25. 8.16 — 9th month	20. 6.17 — 9th month	Died 7.9.17 as a result of an operation.
3403	22. 7.16 — 7th month	1. 8.17 — 9th month	None	Has not aborted or calved normally since the last abortion and is isolated in herd (A).
3334	21. 9.16 — 8th month. Calf alive, but died	16. 8.17 — 4th month	None	(Cow killed on 20.8.17 for inoculation experiments with guinea-pigs.
3040	25. 2.17	Feb., 1918 — 3rd month	None	Still isolated with herd (A).
927	19. 7.16	5.11.17 — 9th month	None	Isolated in herd (A).
316	22. 6.13 — 4th month	None	None	Calved normally 13.5.14; killed 24.12.15.
2208	29.11.15 — 8th month	None	None	Died December, 1915.
777	14. 2.17	None	None	Died January, 1916.
943	30. 4.15	None	None	Calved normally 20.9.16; died —.17.
2736	27. 2.15 — 5th month	None	None	Calved normally 2.1.17; died —.17.
2245	3. 3.15	None	None	Calved normally 19.10.16; died —.17.
2573	Nov., 1914	None	None	Calved normally 1.2.17; isolated in herd (B).
2666	9.11.13	None	None	Killed on —. —. — on account of sterility.
2706	16. 2.15	None	None	Calved normally 17.7.17; isolated in herd (B).
2737	June, 1916	None	None	Has not calved since; isolated in herd (A).
2929	1. 9.15 — 9th month	None	None	Died —. —. —.
3022	19. 3.14	None	None	Died 24.2.16.
3089	16. 1.14	None	None	Died —.2.16.
3103	19.11.16	None	None	Calved normally 13.11.17; isolated in herd (B).
3223	2. 9.15 — 9th month	None	None	Calved normally on 3.11.16 and —.18; isolated in herd (B).
3335	26.12.17	None	None	Isolated in herd (A).
3748	28. 8.17 — 5th month	None	None	Isolated in herd (A).

TABLE II.

Cow.	History.
922	Died on 20.2.16. Did not calve after giving positive reaction.
925	Calved normally 1. 5.15. Died 14.10.15 of Heartwater.
1148	" " 28. 8.16 and 27.12.17.
1200	" " 13. 2.17.
1230	" " 27. 5.15, 28.9.16, and 5.3.18.
1368	Died of Heartwater 3.3.16.
1448	Calved normally 14. 3.17.
1743	" " 17. 5.15 and 3.11.16.
2227	" " 7. 1.17.
2569	" " 21. 9.16.
2566	" " 29. 2.16 and 4.11.17.
2570	" " 29. 9.15 and 24.12.17.
2597	" " 2.10.16.
2664	" " 8. 5.15 and 16.10.17.
2724	" " 30. 9.15 and 20. 9.17.
2772	" " 5.11.17.
2774	" " 25. 8.15.
2777	" " 28. 8.15. Died 26.2.16 of Heartwater.
3082	" " 29. 9.15.
3037	" " 4.11.17.
3023	" " 18. 7.14. Died 23.2.16 of Pneumonia.
3329	" " 25. 2.17.
3409	Died 13.1.16 of Heartwater.
3380	Calved normally 4.11.16.
3374	" " 28. 2.15. Died 27.5.15.
3541	" " 27.10.16. Died 8.11.16 as a result of exposure.
3542	" " 13. 6.17. Died 13.8.17.
3705	Died 29.1.18 of Heartwater.

OBSERVATIONS ON IMMUNITY.

It would appear from our observations here on a large herd of cows, all of which are positive reactors to the agglutination test, that some immunity to Contagious Abortion is acquired by cows in South Africa. Of the infected herd at this laboratory, out of about fifty cows and heifers collected together from different sources, twenty-two have aborted, the remainder having calved apparently normally, a few being barren. The disease in South Africa shows a similar course to the European and American diseases as a rule. When it first attacks a herd a large number will abort, but after about three years abortions are only occasional. Abortion occurs at any time during the period of pregnancy, and there is a marked tendency for the aborting cows in a herd which has been infected for a few years to carry the calves almost the full time or give birth to weak calves. This would also be in agreement with the observations of workers with this disease in other parts of the world. A large proportion of cows which abort do not do so again, but a certain proportion will abort twice, or even three times, as we have had occasion to observe in the laboratory herd. One cow which aborted in three successive years had a live calf, big and strong, at the last calving, but had a retained afterbirth, which was strongly infected. It will be interesting to note whether this cow ever aborts again, as some infected cows undoubtedly give birth to live calves and afterwards abort. A large number of infected cows, as judged by their reaction to the agglutination test, never actually abort at all, though undoubtedly harbouring the abortion bacillus.

Numerous cases occurred here of cows with agglutination titres as high as 1-1000, which showed heavily infected udders at the time of calving in an apparently normal manner. One such cow had, in addition, infection of the uterus, a small piece of the afterbirth being retained and producing lesions on inoculation into guinea-pigs. In a recent publication Schroeder and Cotton state that where a cow at date of calving is reacting positively to serum tests the uterus contains some infection, even if the cow calves in a normal manner. Since the appearance of this publication numerous inoculations have been made with the afterbirths of positively reacting cows, which calved normally, and the results obtained are in agreement with those obtained by Schroeder and Cotton. As has been previously stated, the method of immunization adopted by Stockman, and used on a large scale in England and Scotland, has not yet been introduced into South Africa. The method used by Stockman consists in inoculating uninfected non-pregnant heifers and cows which have aborted before being put to the bull again with virulent cultures of *B. abortus* in large doses subcutaneously. This method would appear to have a good foundation according to certain theories recently brought forward by Schroeder and Cotton in their publication, "Some Facts about Abortion Disease." They state that when the udder is pouring virulent bacilli into the blood, so strong an antibody production may go on that the foetus may be protected at least to some extent. Thus, if a non-pregnant heifer be infected with virulent cultures, a similar immunity is produced.

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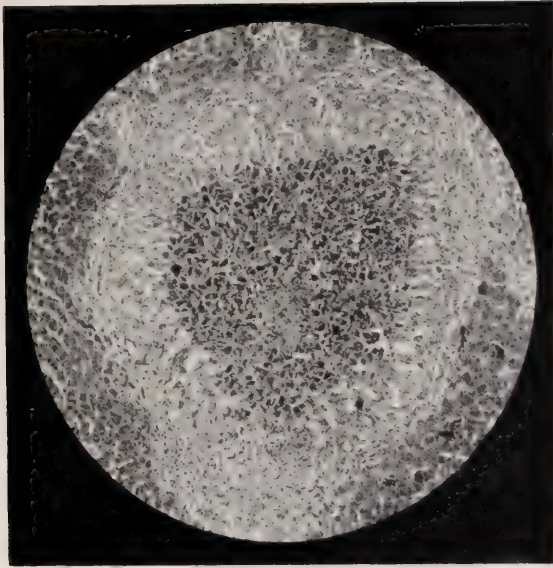


FIG. 1.
Magn. $\times 80$.

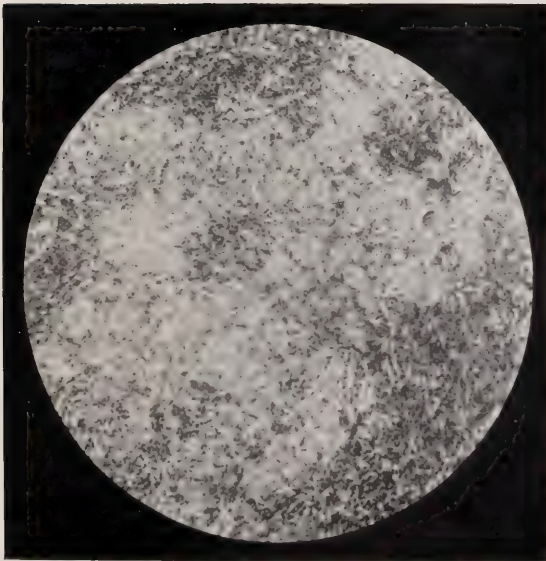


FIG. 2.
Magn. $\times 100$.

Chemotherapy of Haemonchosis in Sheep.

BY

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Chemotherapy of Haemonchosis in Sheep.

(By Dr. FRANK VEGLIA, Veterinary Research Officer, Onderstepoort.)

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INTRODUCTION.

In a previous paper on the "Anatomy and Life History of *Haemonchus contortus*," it was stated that prophylactic measures could play a considerable rôle in the eradication of Wire-worm in sheep, but no preventive method was suggested that definitely met the wants of the practical sheep farmer in South Africa. A combination of prophylactic and therapeutic measures must be used to stamp out or to reduce to a minimum *Haemonchosis* in South Africa. The present paper is devoted to the Chemotherapy of wire-worms in sheep, and the

object has been to find out a drug treatment which will eradicate infection from the animals. In 1912 Sir Arnold Theiler demonstrated experimentally that a single dose of any of the wire-worm drugs then in use in South Africa was not capable of expelling all the parasites from an infected sheep. Ransom arrived at the same conclusion in working with the vermifuges in use in the United States. The present experimental work was started in 1912, setting out from the earlier observations of Sir Arnold Theiler, and attention was chiefly devoted to finding out whether a dose of vermifuge repeated on a number of consecutive days would be likely to free a sheep from wire-worms. The plan of the work has been to infect experimental animals artificially and so obtain material on which drugs could be tested in a systematic and rational manner. It was thought advisable to divide the observations into the four following sections:—

(1) Passage of the drug through the alimentary canal of sheep. (2) Effect of the drug on the wire-worm "in vitro." (3) Effect of the drug on the wire-worm in the host and effect of the drug on the host itself. (4) The effect of dietetic and environmental conditions which come into action during the time of treatment. It is well known that the physiology of the stomach of ruminants is the most complicated of all the domesticated animals, and it is beyond doubt that the effect of a drug on parasites located in the abomasum depends greatly on the state in which the drug enters this division of the stomach. The literature on the passage of drugs, and more generally of solids and liquids, through the stomach of ruminants is rather scanty. The only available paper at the time of these investigations was that of Craig. It was, therefore, found necessary to make a series of observations on the subject, which could give assistance in planning a method of treatment. The chemical analysis of the contents of the alimentary canal of sheep was regarded as being of great importance in determining the fate of drugs when injected. For the valuable assistance given in connection with this part of the work the author has to thank Dr. Green of this Institute, whose own paper upon the fate of ingested and injected arsenic in the sheep should also be consulted (*vide* footnote).^{*} The effect of the drugs on *Haemonchus contortus* "in vitro" was treated somewhat on the lines worked out previously by McFadyean. The purpose was first to ascertain the direct parasitotropic effect of the drug on the helminth outside the body, so as to have a basis of comparison of the effect of the same drug on the worm in the host. In this way the degree of interference with the action of the drugs which might be produced in their passage through the stomach of the sheep could be determined.

Through the study of the drugs "in vitro," it appeared possible to distinguish in the same drug two modes of action, one physical and the other chemical; the relative degree in which these two actions are exercised depending on the concentration of the solution of the drug. From the information so obtained it was possible to explain how various drugs which are the most effective in comparatively strong solution "in vitro" exhibit a relatively slow degree of effectiveness when tested on the infected sheep. In the experiments dealing with

^{*} H. H. Green. "The fate of ingested and injected Arsenic in Sheep, with special reference to Arsenical Treatment of Haemonchosis." Fifth and Sixth Report of the Director of Veterinary Research, Union of South Africa.

the effect of the different drugs on the worms in the host, and on the host itself, the various drugs used were tested separately, and the arsenical compounds were found to be the most effective. Further, it was found experimentally that a single medicine of the series generally used for wire-worm (picric acid, thymol, beta naphthol, bluestone, Cooper's dip, lysol, creosote, gasoline, etc.) is never completely and constantly effective, and that the most successful results are obtained by combined therapy. It is a well recognized principle in Chemotherapy that an ideal anthelmintic should completely destroy the parasites and be not injurious to the host. In the special case of wire-worm infection such a remedy is not known, since the anthelmintics commonly used, if given in a single dose capable of killing all the wire-worms, can prove highly toxic to the host. Bearing these points in mind, an experimental attempt was made to find a remedy, or a combination of remedial agents, satisfying the parasitotropic and organotropic requirements of a practical, and, as far as possible, radical, treatment. The greater part of the present work was carried out on the assumption that a second dose of any given remedy could be administered after a period when the host had recovered from the first dose, but the parasite still remained under its influence. Incidentally, during dosing experiments on the practical field scale, it was observed that the treatment put forward for wire-worms was also effective against the tape-worms of sheep located in the small intestine, so that it is expected that the treatment recommended for the former will be found incidentally useful against the latter. The observation made during the present work that young wire-worms are more susceptible to vermicides than the old ones, suggested a promising way of devising a means of eradication from pasture as well as flock. It was, in fact, experimentally found that sheep submitted to an initial treatment of a double dose of arsenite of soda and bluestone, the drug combination finally selected for general use, are freed from old and young worms. If the same sheep are then treated every successive month with a single dose they are freed from young wire-worms picked up in the pasture during the time elapsing between the two successive treatments. From a theoretical point of view it is believed that after a year of such treatment, not only the flock itself, but also the pasture, ought to be freed from Wire-worm infection.

In the last part of the period during which the present work was carried out (1917) it was found that the practical farmer occasionally passed an unfavourable verdict on the effect of the treatment advocated, by only taking into consideration the question of improvement of the flock after treatment. It was then observed that when all the wire-worms are killed in a treated sheep, other minute helminths frequently present in the abomasum and in the small intestine remain apparently unaffected. These helminths are chiefly of the genus *Trichostrongylus* and produce considerable lesions on the digestive mucosa, with the result that an anæmic sheep so affected is unable to pick up in condition as readily as might be expected.

The *Trichostrongyli* would appear to be of widespread occurrence in sheep in South Africa and would seem capable of producing serious interference with sheep rearing. It is hoped to devote more attention to this parasite in subsequent studies.

A.—THE PASSAGE OF THE DRUG THROUGH THE ALIMENTARY CANAL OF SHEEP.

(a) OBSERVATIONS CONCERNING THE CONTENTS OF THE FOUR DIVISIONS OF THE STOMACH.

Data representing observations undertaken with the object of finding out the contents of the four divisions before and after varying periods of fasting, may now be given:—

Table No. 1.—Stomach Contents of three Lambs, aged 10 months, killed 2½ hours after feeding.

Date Killed.	No. of Lamb.	Contents of				Remarks.
		Rumen.	Reticulum.	Omasum.	Abomasum.	
		c.c.	c.c.	c.c.	c.c.	
17.7.16	9544	5,000	175	Traces	60	Thick liquid.
19.7.16	9591	2,700	225	"	900	Liquid.
24.7.16	9582	1,760	85	"	50	"

Remarks: Rumination was not observed after feeding.

Table No. 2 gives the results of the examination of the stomach contents of 17 lambs, 10 months old, and averaging between 35 and 40 lb. in weight, killed 17 hours and 20 hours after feeding:—

Table No. 2.

Date Killed.	Time of Fasting.	No. of Lamb.	Contents of				Remarks.
			Rumen.	Reticulum.	Omasum.	Abomasum.	
			c.c.	c.c.	c.c.	c.c.	
21.11.16	17 hours	10487	1,940	140	A few	150	
	"	10463	2,000	185	"	129	
	"	10464	2,080	125	"	55	
	"	10467	1,000	129	"	70	
	"	10477	2,000	165	"	100	
23.11.16	"	10468	2,000	100	"	500	
	"	10460	4,800	115	"	480	
	"	10476	3,480	100	"	325	
	"	10475	2,860	105	"	230	
	"	10459	3,120	255	"	525	
24.11.16	20 hours	10472	2,000	100	"	30	Very poor condition. A quantity of sand found in abomasum.
	"	10483	1,000	115	"	135	
	"	10465	1,320	255	"	125	
	"	10462	1,700	130	"	125	
	"	10479	3,800	135	"	115	
25. 7.16	"	9533	2,175	150	Traces	60	Contents of abomasum liquid.
1. 8.16	"	9519	2,000	195	"	60	

Remarks: Normal rumination was observed in the night before death, but was only intermittent on the day of death.

Table No. 3 shows the results of examination of the stomach contents of adult sheep killed two or four hours after feeding:—

Table No. 3.

Date Killed.	No. of Sheep.	Contents of				Remarks.
		Rumen.	Reticulum.	Omasum.	Abomasum.	
		c.c.	c.c.	c.c.	c.c.	
20. 11. 16	10789	2,635	60	—	150	—
	10629	6,235	300	—	270	—
	10236	3,325	175	—	220	—
	10693	5,440	325	—	200	—
	10786	5,000	300	—	315	—
22. 11. 16	10329	3,000	75	—	400	Contents of abomasum liquid.
	10565	2,490	215	—	45	
	10708	4,000	300	—	940	" "
24. 8. 16	8664	4,500	200	70	430	
	9068	5,150	300	105	290	
3. 6. 16	8406	5,000	195	50	325	Sheep in good condition.
31. 3. 16	8710	2,700	100	Traces	120	Liquid contents and mucus in abomasum.
	—	2,400	200	Traces of hard material	100	" "
	8199	3,700	300	—	200	Sheep in very good condition.
27. 6. 16	8111	1,340	510	100	35	" "
19. 6. 16	9238	3,000	200	Traces	25	Sheep rather poor.
5. 1. 17	10641	1,500	100	50	300	

Remarks: Rumination was normal during night previous to death, but was only intermittent after the last meal. Some were ruminating when killed.

Table No. 4 shows the result of examination of the stomach contents of adult sheep killed at different intervals after feeding:—

Table No. 4.

Date Killed.	No. of Sheep.	Interval.	Contents of				Remarks.
			Rumen.	Reticulum.	Omasum.	Abomasum.	
			c.c.	c.c.	c.c.	c.c.	
14.12.16	10703	17 hours	2,200	100	—	80	—
	10736	"	2,000	160	—	250	—
30. 6.16	8449	"	2,615	335	55	110	—
	7717	"	4,205	325	60	165	—
3. 7.16	8620	"	3,000	510	185	335	—
	9062	"	2,485	240	25	105	—
29.11.16	10658	20 hours	3,425	150	—	315	—
	10674	"	5,560	165	—	270	—
	10762	"	3,480	125	—	295	—
	10725	"	3,480	125	—	165	—
6.12.16	10668	"	3,100	265	—	360	—
	10567	"	2,000	275	—	250	—
	10714	"	2,250	300	—	150	—
	10705	"	2,300	450	—	320	—
	10771	"	2,160	270	—	340	—
29.11.16	10712	24 hours	4,800	425	—	265	—
	10732	"	4,840	350	—	200	—
7. 4.16	7713	17 hours	1,000	80	Traces	100	Sheep was fat but anæmic.
	8511	"	1,000	105	—	55	Sheep showed poverty; diarrhoea; anæmia.
1. 4.16	8204	"	2,600	130	Soft traces	75	Sheep was poor and anæmic.
11. 4.16	8325	"	2,350	62	Traces	313	Sheep was in fair condition.
2. 5.16	8133	"	2,000	100	"	120	
17. 1.17	10731	"	3,000	300	50	100	Sheep was in good condition.
21. 6.16	9427	"	700	60	Traces	175	Sheep showed poverty and anæmia.
18. 4.16	8069	12 hours	2,800	90	"	300	Sheep was in good condition.
22. 6.16	9434	17 hours	875	65	A few c.c.	A few c.c.	" "
23. 6.16	9416	"	1,750	175	Traces	350	" "
29.12.16	9725	19 hours	1,500	100	50	310	" "
1. 2.17	10638	17 hours	3,320	118	50	198	" "

Remarks: Normal rumination was observed in the night before death, but was only intermittent on the day of death.

Conclusions.

1. It appears that the contents of the rumen and the reticulum in sheep killed soon after feeding is in general not much greater than those starved for a period varying from 17 to 24 hours. In the case of the abomasum there may be a marked difference.

2. In closely observing the functions of feeding and ruminating, it appeared that in sheep that were being kept without food rumination was marked after a feed, chiefly late in the evening, but became more and more remittent as the starvation period lengthened. Seventeen hours after feeding rumination is practically stopped. This observation was made in the stables of the laboratory where, during the day, sheep are seldom in that quiet condition in which rumination is performed.

3. During the starvation periods of from 17 to 24 hours the semi-solid contents of the rumen maintain an average bulk, and it is only in prolonged starvation, effected either by the intervention of man or by pathological conditions, that the bulk of the rumen decreases in any marked proportion.

(b) AGENTS USED IN THE EXPERIMENTS ON THE "PASSAGE OF DRUGS."

Two series of experiments were carried out, adopting two different methods of investigation. In the first series staining agents were administered to the sheep, which could be detected in the alimentary tract by their particular colour. In the second series arsenical compounds were administered to the sheep and their presence or their concentration in the various stomachs and intestines detected by chemical analysis.

First Series.—Two substances were used for the experiment:—

1. A powder not soluble in contact with the stomach and intestinal content and easily detected in small fractions.

2. A soluble powder which when administered as such or in solution would stain the contents of the alimentary canal in a conspicuous manner from the rumen to the rectum.

To begin with, charcoal and French chalk were used, but their effect was not sufficiently conspicuous. Methylene blue was also tested, but this stain was found to lose its typical appearance in contact with the juices of the alimentary tract. Finally, magenta, either in powder or in solution, and aluminium powder, were used with most satisfactory results. The magenta stain is easily detected in the ingesta, even when in very weak solution, and for the first 24 hours after administration the liquid and solid contents of the alimentary canal are well coloured. The colour is unaltered except in rare cases; e.g. in the abomasum and large intestine, when a vinous colour is noted, although the concentration is comparatively high. Twenty-four hours after administration the liquids are of normal colour and only the solid particles are stained red; a reason for this will be suggested in discussing the various experiments separately. Aluminium powder is practically not dissolved by the digestive products. On account of its light character it is easily mixed with the ingesta and does not deposit in the reticulum, abomasum or colon, as in the case of some other metallic powders. Furthermore its shiny appearance is not altered by the digestive products. To trace the course of soluble and insoluble powders in the sheep, one teaspoonful of powder consisting of two-thirds of magenta and one-third of aluminium is sufficient for the purpose. In tracing the course of liquid a constant quantity of 140 c.c. was generally administered, this being the average amount suggested by American writers in dosing sheep with creosote solution.

Pills, made up by mixing the staining powder with saturated watery solution of gum acacia and allowing to dry in the open, were also tried. To the staining powder or pill a certain amount of bluestone and sodium arsenite was occasionally added for the purpose of ascertaining if stimulant drugs take more or less time to pass the abomasum than the inert magenta and aluminium powders. A pill of approximately 1 cm. diameter was found serviceable for the amount of bluestone and arsenite or Cooper's dip required for dosing adult sheep.

Second Series.—Commercial arsenite of soda plus bluestone were administered together taking 125 mgs. of arsenite as chemically equivalent to 500 mgs. of Cooper's dip. At prearranged times, after dosing, the sheep were killed and the whole alimentary tract passed for analysis to Dr. Green, who, as already mentioned, dealt with this section of the work.

(c) POSITION OF THE SHEEP DURING DOSING AND ITS INFLUENCE ON THE PASSAGE OF THE DRUGS.

The position in which the sheep were held during administration of the dose is dealt with in more detail later, but it is considered advisable to refer to it at this point. It is generally believed that the course of the drug is modified to a great extent according to whether the sheep is kept standing on its four legs or placed on its haunches when the dose is administered. Two series of observations, detailed later, were made, as a result of which it may be affirmed that the position of the sheep during dosing does not appreciably or consistently affect either the mode of passage of the drug through the stomach system, or its effect upon the wire-worms.

(d) PRESENCE OF DRUGS IN THE DIGESTIVE TRACT, AS GAUGED BY COLOURING AGENTS.

In this section the word "drug" means powder or colouring agent given alone or admixed with sodium arsenite and bluestone. Except when otherwise mentioned, the drug was "administered." The cases in which voluntary intake was effected by licking, or drinking in solution, are given in an appendix. In speaking of stomach contents, the contents of all four pouches is meant. The observations were made by dosing sheep and killing at various periods thereafter. The results are detailed seriatim and arranged according to the length of time elapsing between dosing and killing. In each case the number of the sheep is given, and following this the number of the Table in which reference to the figures for stomach contents may be found. Special attention was paid to the physical state of the drug, to the position of the sheep during administration, to the repletion of the stomach when the sheep was dosed, and to the condition of health and nutrition.

(1) *Sheep Killed from 3 Minutes to 10 Minutes after Dosing.*

1st (3 min. and 8 mins.).—No. 8406 Merino sheep, 8-tooth, good condition (Table No. 3). 3rd June, 1916, 11 a.m., dosed with magenta powder. 11.3 a.m., administration of 100 c.c. of saturated watery solution of magenta (on its haunches); 11.8 a.m., animal killed.

Result.—Magenta stain is present in the reticulum and anterior dorsal blind sac of rumen extending slightly backwards along the walls of the rumen, and well limited in the ventral part by the pila cranialis. Slight pink coloration in sulcus omasi to the ostium omaso-abomasum. Aluminium powder is present in the mouth and oesophagus, and distributed in the stomach as already described for the magenta solution. The rumen content forms a homogeneous, fairly consistent, ball of ingesta.

2nd (5 mins.).—No. 10638, Merino, 8-tooth (Table No. 4), starved. 1st February, 1917, 9 a.m., sheep dosed (on its haunches) with 10 c.c. of magenta solution by squirting it into the mouth by means of a Pravaz syringe; killed, 9.5 a.m.

Result.—Magenta staining over a patch as large as a hand on the surface of the solid ingesta of anterior dorsal blind sac of rumen just at the opening of the cardia. Contents of the reticulum also red. No traces of red in omasum, abomasum, or in the rumen.

3rd (5 mins. and 15 mins.—pills).—No. 9544, Merino lamb, 10 months, good condition (Table No. 1); not starved. 17th July, 1916, 9 a.m., dosed with a pill of magenta and aluminium powder 5 mm. in diameter; the pill was masticated; 9.10 a.m. (on its haunches) a second pill equal to the first one, was given; this was swallowed whole; 9.15 a.m., animal killed.

Result.—First pill still in the mouth adhering to the left molar teeth. Pink coloration is also present in the reticulum content and in the sulcus omasi. Traces of pink coloration are present in the abomasum. The second pill is still undissolved in the anterior blind sac of the rumen.

4th (10 mins.).—No. 10771, Merino, 6-tooth, fair condition; not starved. 14th December, 1916, 4 p.m., dosed (on its haunches) with 10 c.c. of magenta by squirting into the mouth with Pravaz syringe; 4.10 p.m., sheep killed.

Result.—Anterior dorsal blind sac of rumen and the reticulum show homogeneous red coloration. A few red patches are also present on the dorsal surface of the rumenal content, which is semi-solid and forms a fairly consistent ball of ingesta.

5th (10 mins.—animal charcoal).—No. 8204, Merino sheep, 4-tooth, animal poor (see Table No. 4 for contents of stomach); starved 17 hours. 1st April, 1916, 9 a.m., dosed (on its haunches) with a teaspoonful of charcoal in fine powder; 9.10 a.m., animal killed.

Result.—Charcoal found at the bottom of the reticulum and in the contents of anterior dorsal blind sac of the rumen. Some traces are present in the omasum and still fewer in the abomasum.

(2) Sheep Killed 15 Minutes after Dosing.

6th.—No. 9693, Merino, 8-tooth, condition poor. 30th August, 1916, 9.30 a.m., dosed (standing) with 140 c.c. of saturated magenta solution; 9.45 a.m., animal killed.

Result.—Deep red coloration in anterior dorsal blind sac of rumen and in reticulum. Large, well defined red patches present in the rumenal mass of ingesta, chiefly at the periphery. Red coloration also present in sulcus omasi. Very slight pink coloration in the cardiac region of abomasum.

7th.—No. 9435, Merino, 6-tooth, poor condition; not starved. 26th August, 1916, 9.50 a.m., dosed with 140 c.c. of saturated magenta solution (animal standing); 10.5 a.m., animal killed.

Result.—Intense red coloration of the reticulum, anterior dorsal blind sac of rumen, sulcus omasi and abomasum.

8th.—No. 9740, Merino, 4-tooth, poor condition; starved 17 hours. 11th September, 1916, 3 p.m., dosed with 140 c.c. of saturated magenta solution (animal standing); 3.15 p.m. animal killed.

Result.—Red coloration of sulcus omasi and near the omaso-abomasal orifice region of abomasum. NOTE.—The rumen contents are rather scanty and pultaceous.

9th.—No. 9418, Merino, 6-tooth, in fair condition; not starved. 25th August, 1916, 11.15 a.m., dosed with 140 c.c. of saturated magenta solution (animal standing); 11.30 a.m., animal killed.

Result.—Red colour intense in anterior dorsal blind sac of rumen and in reticulum. Isolated patches at the periphery of rumenal ingesta. Pink colour of sulcus omasi and a very few red points in the abomasum.

10th.—No. 8481, Merino, 8-tooth, in fair condition; not starved. 26th August, 1916, 11 a.m., dosed with 140 c.c. of saturated magenta solution (animal standing); 11.15 a.m., animal killed.

Result.—Anterior dorsal blind sac of rumen and reticulum dark red. Large red patches on the periphery of the rumenal contents. Sulcus omasi, red. No colouration in the abomasum.

11th.—No. 9711, Merino, 6-tooth, fair condition; not starved. 13th September, 1916, 3 p.m., dosed (on its haunches) with 140 c.c. of saturated magenta solution; 3.15 p.m., animal killed.

Result.—Anterior dorsal blind sac of rumen and reticulum intense red. Ruminal contents show some well-defined lumps of intense red ingesta. No coloration in either omasum or abomasum.

12th.—No. 9752, Merino, 8-tooth, poor condition; not starved. 13th September, 1916, 4 p.m., dosed with 140 c.c. of magenta solution (animal standing); 4.15 p.m., animal killed.

Result.—As with No. 9711.

13th.—No. 9664, Merino, 8-tooth, poor condition; not starved. 14th September, 1916, 9 p.m., dosed (on its haunches) with 140 c.c. of magenta solution; 9.15 p.m., animal killed.

Result.—Anterior dorsal blind sac of rumen and reticulum shows pink colour. Traces of red in the rumen, omasum and abomasum; intense red throughout contents.

14th.—No. 9515, Merino lamb, 11 months, condition fair; not starved. 27th August, 1916, 10 a.m., dosed (on its haunches) with 70 c.c. of magenta solution; 10.15 a.m., animal killed.

Result.—Anterior dorsal blind sac of rumen and reticulum intense red. Numerous small red lumps in the rumen; omasum red; abomasum traces of red.

15th.—No. 10491, Merino lamb, 11 months, condition fair; not starved. 5th October, 1916, 10 a.m., dosed (on its haunches) with 10 c.c. of magenta solution with a Pravaz syringe; 10.15 a.m., animal killed.

Result.—Anterior dorsal blind sac of rumen and reticulum red. Rumen: small red lumps scattered in the periphery of the contents. No coloration in the omasum or abomasum.

16th.—No. 10753, Merino, 6-tooth, condition good (for stomach contents see Table No. 4); starved. 6th February, 1917, 8.45 a.m., dosed (on its haunches) with a teaspoonful of magenta powder; 9 a.m., sheep killed.

Result.—Anterior dorsal blind sac of rumen and the reticulum are red. Red lumps scattered in rumenal contents, but not in the ventral sac. Sulcus omasi red; abomasum not coloured.

17th.—No. 9416, Merino, 6-tooth, in good condition; 9 a.m., dosed with pill; 9.15 a.m., killed.

Result.—Pill was found in the anterior dorsal blind sac of rumen.

(3) *Sheep Killed 20 or 30 Minutes after Dosing.*

18th (20 mins.).—No. 8111, Merino, 6-tooth, good condition; not starved. 27th June, 1916, 4 p.m., dosed (on its haunches) with pill of magenta 1 cm. in diameter, slightly greased with vaseline; the pill was swallowed whole; 4.20 p.m., animal killed.

Result.—Rumen and reticulum contents homogeneously red; omasum and abomasum not coloured.

19th.—No. 8918, Merino, 6-tooth, good condition; not starved. 28th June, 1916, 4 p.m., dosed with a pill of 100 mg. sodium arsenite, 500 mg. of bluestone, 25 mg. of magenta; diameter, 1 cm.; 4.20 p.m., animal killed.

Result.—Anterior dorsal blind sac of rumen and reticulum are red; slight red coloration in sulcus omasi; abomasum not coloured.

20th (30 mins.).—No. 8664, Merino, 8-tooth, condition fair; not starved (see Table No. 3 for stomach contents). 24th August, 1916, 9 a.m., dosed (on its haunches) with a pill as for No. 8918; 9.30, animal killed.

Result.—Anterior dorsal blind sac of rumen and the reticulum are coloured red; red lumps on the periphery of the rumenal mass; pink coloration in sulcus omasi; no traces of coloration in the abomasum.

(4) *Sheep Killed 1 Hour after Dosing.*

21st.—No. 8943, Merino, 6-tooth, good condition; not starved. 26th August, 1916, 9.30 a.m., dosed with 140 c.c. of saturated magenta solution (animal standing); 10.30 a.m., killed.

Result.—Anterior dorsal blind sac of rumen and reticulum, red coloration; rumen contents consist of mixed stained and unstained lumps; pink colour in sulcus omasi, and traces of pink colour in abomasum.

22nd.—No. 8614, Merino, 8-tooth, condition fair; starved. 1st April, 1916, 10 a.m., dosed (on its haunches) with one teaspoonful of French chalk stained with methylene blue; 11 a.m., animal killed.

Result.—Small clusters of chalk and slight blue discoloration of the ingesta are seen in the anterior dorsal blind sac of the rumen and in the reticulum; a small amount was in the rest of the rumen; omasum: traces of chalk in sulcus omasi, none in abomasum.

23rd.—No. 8449, Merino, 6-tooth, condition good (see Table No. 4 for stomach contents); sheep starved. 30th June, 1916, 9 a.m., dosed (on its haunches) with a pill of magenta and aluminium powder, having a diameter of 1 cm.; the pill was well swallowed. 10 a.m., sheep killed.

Result.—In the rumen and reticulum the magenta stain and aluminium powder are homogeneously mixed with the ingesta; sulcus omasi is pink in colour.

24th.—No. 8364, Merino, 6-tooth, fair condition; not starved. 23rd August, 1916, 9 a.m., dosed (on its haunches) with a pill composed of sodium arsenite 125 mg., bluestone 400 mg., magenta stain 100 mg.; 10 a.m., killed.

Result.—Anterior dorsal blind sac of the rumen and reticulum homogeneous red coloration; rumen: red patches on the periphery of the contents; omasum: sulcus omasi red in colour; abomasum: very few particles of aluminium just past the orifice omaso-abomasicum.

25th.—No. 7713, Merino, 4-tooth, anæmic; fat; starved (see Table No. 4 for stomach contents). 7th April, 1916: 9 a.m., dosed (on its haunches) with a teaspoonful of aluminium powder mixed with sugar; 10.30 a.m., sheep killed.

Result.—Nearly the whole of the powder is homogeneously mixed with the contents of the rumen and reticulum; a very small amount is mixed with the abomasum contents; no traces of powder in the small intestine.

26th.—No. 10676, Merino, 8-tooth, condition fair (Table No. 4); starved. 24th January, 1917, 12 noon, dosed (on its haunches) with one teaspoonful of magenta; 2 p.m., sheep killed.

Result.—Mouth: the main portion of the magenta is reduced to paste between the left cheek and the molar teeth. A fair amount is also stuck to the dorsal surface of the tongue. The lips of the mouth are also red and wet with saliva. Pharynx and oesophagus are not coloured. Patches of pink in the rumen; partial red coloration in the blind sac. Reticulum, omasum, and abomasum not coloured.

This is a case in which the drug in the mouth has been retained unusually long. It may be said, however, that arsenic and bluestone are more irritant than magenta powder, and that sheep would never keep such in the mouth for so long a time.

(5) *Sheep Killed Two Hours after Dosing.*

27th.—No. 8620, 6-tooth, condition good (see Table No. 4 for stomach content); starved. 3rd July, 1916, 9 a.m., dosed (on its haunches) with a pill of aluminium and magenta 7 mm. in diameter; pill was masticated and swallowed slowly; 11 a.m., animal killed.

Result.—Homogeneous intense red coloration of reticulum and anterior blind dorsal sac of rumen; rumen homogeneous red coloration; omasum, pink coloration in sulcus omasi; abomasum, slight pink coloration and particles of aluminium in fundus region; no traces of red or aluminium in pyloric region.

28th.—No. 9668, Merino, 6-tooth, condition poor; not starved. 15th September, 1916, 9 a.m., dosed (on its haunches) with a pill of magenta, 9 mm. in diameter; 11 a.m., animal killed; only traces of pink colour are found in the ostium reticulo-omasicum; apparently the pill was not swallowed, but was spat out after a short stay in the pharynx.

29th.—No. 9062, Merino, 6-tooth, condition poor (for contents of stomach see Table No. 4); starved. 3rd July, 1916, 9 a.m., dosed (on its haunches) with a pill of magenta and aluminium 7 mm. in diameter; the pill was well swallowed; 11 a.m., sheep killed.

Result.—Reticulum, homogeneously red; rumen, homogeneously pink in colour; omasum, red in sulcus omasi; abomasum, intense red; jejunum, pink more intense than rumen; ileum, no traces of colour. It appears that the pill was melted in the reticulum from which the solution passed rather concentrated to the abomasum. The slight homogeneous staining of the rumen contents shows that only small quantities of colouring material passed from the reticulum to the rumen.

30th.—No. 8886, Merino, 8-tooth, fair condition; not starved. 14th September, 1916, dosed (on its haunches) with a pill of magenta and 100 mg. of sodium arsenite of 9 mm. diameter; 11 a.m., sheep killed.

Result.—Rumen and reticulum intense red; omasum: traces of red in sulcus omasi; abomasum: no traces of red.

31st.—No. 8947, Merino, 6-tooth, fair condition (see Table No. 4 for stomach contents); not starved. 23rd August, 1916, dosed (on its haunches) with pill of 100 mg. arsenite of soda, 400 mg. bluestone, and 100 mg. magenta; 1 c.m. in diameter; 11 a.m., sheep killed.

Result.—Rumen, reticulum, sulcus omasi, red; abomasum, pink in colour; jejunum, traces of pink coloration.

32nd.—No. 9612, Merino, 8-tooth, poor condition; not starved. 19th September, 1916, 9 a.m., dosed (on its haunches) with a pill of 100 mg. of sodium arsenite, 400 mg. bluestone, and 100 mg. of magenta; diameter of pill, 9 mm.; 11 a.m., sheep killed.

Result.—Rumen, pill not completely dissolved; contents intense red; reticulum, intense red; omasum, red; abomasum, traces of pink coloration.

33rd.—No. 8325, Merino, condition fair; starved (see Table No. 4 for stomach contents). 11th April, 1916: 9 a.m., dosed (on its haunches) with Cooper's dip 500 mg., bluestone 500 mg., plus magenta 500 mg., as a mixed powder; 11 a.m., sheep killed.

The stomachs were passed on to the Biochemical Laboratory. The distribution of magenta was as follows:—Rumen and reticulum, homogeneous red coloration; omasum, sulcus omasi intense red; abomasum, homogeneous intense red.

34th.—Lamb, 4 months, condition poor; not starved. 22nd September, 1916, 9 a.m., dosed (on its haunches) with a pill of magenta 9 mm. in diameter; 11 a.m., lamb killed.

Result.—Rumen and reticulum, intense red; omasum and abomasum show no traces of red. To explain the slow process of the colouring material it may be said that the lamb was very weak, and thus the rumenal function probably impeded.

(6) *Sheep Killed Three Hours after Dosing.*

35th.—No. 8611, 6-tooth Merino, poor (for stomach contents see experiment No. 4); starved. 7th April, 1916, dosed (on its haunches) with a teaspoonful of sheep fat stained with scharlach. The substance was swallowed slowly; 12 noon, sheep killed.

Result.—Rumen homogeneously red; reticulum, omasum, and abomasum, intense red; jejunum, red; ileum, slightly red; colon, numerous patches of red; small colon, not coloured.

38th.—No. 7717, Merino, 6-tooth, poor (for stomach contents see Table No. 4); starved. 30th June, 1916, 9 a.m., dosed (on its haunches) with a pill of aluminium and magenta, 1 cm. in diameter; the pill was masticated; 11 a.m., sheep killed.

Result.—Rumen, reticulum, omasum, pink in colour; aluminium powder homogeneously distributed. Abomasum, red in colour and aluminium homogeneously distributed. Jejunum, homogeneous pink coloration. Ileum, no traces of magenta.

39th.—No. 9694, Merino, 6-tooth, fair condition; not starved. 28th September, 1916, 9 a.m., dosed (on its haunches) with pill of 9 mm. in diameter of sodium arsenite, 100 mg.; bluestone, 400 mg.; magenta, 125 mg.; the pill was well swallowed; 12 noon, sheep killed.

Result.—Reticulum and anterior dorsal blind sac of rumen, intense red; rumen, lumps of red; omasum, red; abomasum, traces of red.

40th.—No. 9691. Merino, condition poor; not starved. 19th September, 1916, 9 a.m., dosed on its haunches with a pill as with Sheep No. 9694; the pill was well swallowed; 12 noon, sheep killed.

Result.—Rumen, reticulum, omasum, abomasum, homogeneously red; jejunum, traces of pink colour.

(7) *Sheep Killed Four Hours after Dosing.*

41st.—No. 8133, Merino, 6-tooth, fair condition (for stomach contents see Table No. 4); starved. 2nd May, 1916, 10 a.m., dosed (on its haunches) with Cooper's dip, 500 mg.; bluestone, 500 mg.; magenta, 100 mg., in powder form; 2 p.m., sheep killed. The stomachs were passed to the Biochemical Laboratory. The distribution of magenta was as follows:—Rumen and reticulum homogeneously red; sulcus omasi marked red; abomasum, intense red; jejunum, traces of red.

42nd.—No. 9731, Merino, 8-tooth, fair condition; not starved. 28th September, 1916, 9 a.m., dosed (on its haunches) with a 9 mm. pill of sodium arsenite 100 mg.; bluestone, 400 mg.; magenta 125 mg.; 1 p.m., sheep killed.

Result.—Reticulum, rumen, omasum, homogeneous red colour; abomasum, pink in colour; small intestine, no traces of colour.

43rd.—No. 9715, Merino, 8-tooth, in poor condition; not starved. 5th October, 1916, 11 a.m., dosed on its haunches with a pill as No. 9731; 2 p.m., sheep killed.

Result.—Rumen, reticulum, omasum, homogeneous red colour; abomasum, pink colour; jejunum, numerous traces of pink colour; ileum, no traces. Sheep was seen chewing at 12 noon; this can explain the rapid progress of the drug in the intestine.

44th.—No. 10731, Merino, 8-tooth, condition good; starved (for stomach contents see Table No. 4). 17th January, 1917, 10 a.m., dosed (on its haunches) with a teaspoonful of magenta and aluminium in powder form; 2 p.m., sheep killed.

Result.—Rumen, reticulum, omasum, abomasum, homogeneous red; jejunum, homogeneous red—less marked; ileum homogeneous pink colour up to the ileo-caecal valve.

(8) *Sheep Killed Five Hours after Dosing.*

45th.—No. 9238, Merino, 8-tooth, poor condition (for stomach contents see Table No. 3); not starved. 19th June, 1917, 9 a.m., dosed with half a teaspoonful of aluminium; 9.15 a.m., dosed with half a teaspoonful of magenta (on its haunches); left without food or water until 12 noon; at 12 noon it was chewing; 2 p.m., sheep killed.

Result.—Contents of rumen, reticulum, abomasum, jejunum, ileum, homogeneously stained with red and with the aluminium powder homogeneously distributed. Caecum and large colon, pink coloration. Small colon, no traces of red coloration.

46th.—No. 9728, Merino, 8-tooth, condition good; not starved. 29th August, 1916, dosed (on its haunches) with a 9 mm. pill of Cooper's dip, 100 mg.; bluestone, 400 mg.; magenta, 100 mg.; 2 p.m., sheep killed.

Result.—Rumen, reticulum, abomasum, homogeneously red; jejunum, traces of red; ileum, no traces of red. This case shows that if the sheep is weak and the rumen reduced in contents, as happens in advanced cases of haemonchosis, the drug makes slow progress in the digestive canal.

47th.—No. 10729, Merino, 8-tooth, condition good; not starved. 30th January, 1917, 9 a.m., dosed (on its haunches) with one teaspoonful of magenta powder; 9.15 a.m., dosed (on its haunches) with one teaspoonful of aluminium powder and a little sodium chloride; 2 p.m., sheep killed.

Result.—Rumen and reticulum, pink coloration of the solid and liquid contents; a few particles of aluminium; omasum, red in colour; a small quantity of moist magenta and aluminium is present on the reticulo-omasal orifice; abomasum, solid and liquid contents red in colour and with a fair amount of aluminium; jejunum, contents of a well-marked pink, but with less aluminium than in the abomasum; ileum, deep red coloration of solid and liquid contents, aluminium powder very thick; large intestine, traces of red at the level of the ileo-caecal valve.

(9) *Sheep Killed Ten Hours after Dosing.*

48th.—No. 9427, Merino, 6-tooth, condition fair (for stomach contents see Table No. 4); starved. 21st June, 1916, 9 a.m., dosed (on its haunches) with a teaspoonful of aluminium; 9.12 a.m., dosed with a teaspoonful of magenta; 7.15 p.m., sheep killed.

Result.—Reticulum, dark red; rumen and omasum, homogeneously red; abomasum, pink coloration; jejunum, uncoloured. The aluminium powder was found all along with the magenta stain.

(10) *Sheep Killed Twenty-four Hours after Dosing.*

49th.—No. 9591, Merino lamb, 10 months old, condition fair (for stomach contents see Table 1); starved. 19th July, 1916, 9 a.m., dosed (on its haunches) with one teaspoonful of magenta and aluminium in powder form. 20th July, 1916: the droppings passed out in the early hours of the morning were stained pink in colour. This case shows that in twenty-four hours the drug can be passed with the faeces even when there is no diarrhoea.

50th.—No. 9582, Merino lamb, 10 months old, fair condition; starved (for stomach contents see Table No. 4). 19th July, 1916, 9 a.m., dosed (on its haunches) with one teaspoonful of magenta and aluminium powder. 20th July, 1916, faeces are pultaceous and show marked red colour. Diarrhoea observed.

51st.—No. 8969, Merino, 4-tooth, condition good (for stomach contents see Table No. 4); starved. 18th April, 1916, 9 a.m., dosed (on its haunches) with sodium arsenite; 105 mg.; bluestone, 500 mg.; magenta, 100 mg.; 2 p.m., given green food. 19th April, 1916, 9 a.m., sheep killed (stomach and intestinal contents passed to the Biochemical Laboratory). Distribution of magenta was as follows:—Rumen, reticulum, omasum, abomasum, jejunum, all coloured homogeneous intense red.

52nd.—No. 9434, Merino, 6-tooth, condition rather poor (for stomach contents see Table 4); starved. 21st June, 1916, 9 a.m., dosed with a teaspoonful of aluminium powder; 9.15 a.m., dosed (on its haunches) with a teaspoonful of magenta powder; 2.4 p.m., given green food. 22nd June, 1916, 9 a.m., sheep killed.

Result.—Aluminium and magenta are equally mixed in the various portions of the alimentary tract. Rumen, reticulum, deep red in colour, and contain the greater part of the magenta homogeneously mixed with the food; omasum, red in sulcus omasi; abomasum,

red, but not very marked; small intestine, pink colour; large intestine, slight pink coloration; rectum, red colour detected in faeces.

53rd.—No. 9416, Merino, 6-tooth, condition good (for contents of stomach see Table No. 4); starved. 21st June, 1916, 9 a.m., dosed with a teaspoonful of aluminium; 9.15 a.m., dosed with a teaspoonful of magenta; 2 p.m., given green food. 22nd June, 1916, at 9 a.m., dosed with a teaspoonful of aluminium; 9.15 a.m., dosed with a teaspoonful of magenta (the sheep was dosed on its haunches); faeces are red in colour and contain aluminium; 2 p.m., green food. 23rd March, 1916, dosed with a pill of sealing wax 1 cm. in diameter; 9.15 a.m., sheep killed.

Result.—Pill is found in the anterior dorsal blind sac of rumen: rumen, reticulum, abomasum, and small intestine, homogeneously red; large intestine, very dark red; rectum, red faeces.

54th.—No. 3725, Merino, 8-tooth (for stomach contents see Table No. 4). 28th December, 1916, 9 a.m., dosed with sodium arsenite, 150 mg.; bluestone, 500 mg.; magenta, 100 mg.; 2 p.m., green food. 29th December, 1916, sheep killed.

Result.—The distribution of magenta was as follows:—Rumen, intense red; contents of reticulum, omasum, abomasum, jejunum, and ileum, all red in colour; traces of red in the colon.

(11) *Sheep Killed Forty-eight Hours after Dosing.*

55th.—No. 9591, Merino lamb, 10 months, condition fair; starved (for stomach contents see Table No. 4). 19th July, 1916, 9 a.m., dosed with one teaspoonful of magenta and aluminium powder; 2 p.m., green food. 20th July, 1916, faeces pink in colour; 9 a.m., dosed again with a teaspoonful of magenta and aluminium (sheep dosed on its haunches). 22nd July, 1916, 9 a.m., killed.

Result.—Rumen and reticulum, deep red with a lot of aluminium; omasum, pink in colour; abomasum, pink colour hardly detected, aluminium rare; small intestine and large intestine, traces of pink colour, aluminium particles very rare. Faeces not stained with magenta, but traces of aluminium could be detected.

56th.—No. 10641, Merino, 8-tooth, condition fair (for stomach contents see Table No. 3). 3rd January, 1917, 9 a.m., dosed with sodium arsenite, 150 mg.; bluestone, 500 mg.; magenta, 1 g. 4th January, 1917, food and water as usual. 5th January, 1917, sheep killed. The distribution of magenta was as follows:—Rumen and reticulum, pink in colour; faint pink coloration from omasum to ileum; colon, red. Droppings were found red from the 24th hour after dosing.

(12) *Sheep Killed Three Days after Dosing.*

57th.—No. 10763, Merino, 8-tooth, condition fairly good (see Table No. 4); starved. 23rd July, 1917, 9 a.m., dosed (on its haunches) with sodium arsenite, 150 mg.; bluestone, 500 mg.; in powder form; 9.15 a.m., dosed with a teaspoonful of magenta powder;

2-4 p.m., green food. 24th January, 1917, same treatment as on previous day. 25th January, 1917, 9 a.m., food and water as usual. 27th January, 1917, killed.

Result.—Solid contents of the abomasum and small intestine stained pink; fluid contents of normal colour; contents of the large intestine red. Solid rumen contents partly stained; liquid contents of normal colour.

This case shows that magenta stain in the stomachs of the sheep is partly kept in solution and partly fixed by the solid particles of food. It does not follow, of course, that solid arsenical compounds would behave in the same way. The magenta in solution disappears from the stomach within the first 48 hours after dosing. After three days only the large intestine shows magenta.

(13) *Sheep Killed Four Days after Dosing.*

58th.—No. 9582, Merino lamb, 10 months, condition fair (for stomach contents see Table No. 1). 19th July, 1916, 9 a.m., dosed with one teaspoonful of magenta and aluminium powder. 20th July, 1916, 9 a.m., faeces red colour (compare Experiment No. 50); diarrhoea observed. 24th July, 1916, faeces practically normal; lamb killed.

Result.—Rumen, reticulum, omasum, red in colour; abomasum, pink; jejunum, slight pink coloration; large intestine, red colour more marked than in rumen; marked concentration of aluminium; faeces red in colour.

This case differs considerably from the previous one, as the magenta remained in the alimentary canal for a longer time. The fact that the lamb showed digestive disorders might account for this.

(14) *Sheep Killed Nine Days after Dosing.*

59th.—No. 9519, Merino lamb, 10 months, condition good (for stomach contents see Table No. 2); not starved. 23rd July, 1916, 9 a.m., dosed (on its haunches) with a teaspoonful of magenta and aluminium. 31st July, 1915, faeces are red in colour. 1st August, 1916, lamb killed.

Result.—Numerous red coloured food particles in the reticulum and abomasum; small intestine, no traces of red coloration; large intestine; pink coloration of the contents; faeces, red in colour.

Notes on the Rumination observed in the Dosed Sheep.

1st.—The first 25 sheep, which were killed within the first hour after the administration of the drug, did not ruminate.

2nd.—For the sheep which were killed from 1½ to 5 hours after dosing, it may be assumed with safety that the sheep which were

No. of Sheep.	Time after Dosing.	Substance Ad- ministered	Form of the Sub- stance.	Ru- men.		Reticu- lum.	Omasum.	Abomasum	Jejunum.	Ileum.	Large Intes- tine.	
				1	2						3	4
7713	hours. 1½	Al.	Powder	==	==	==		==				
8620	2	Al. Mag.	Pill-mas- ticated	==	==	==				
9668	2	Mag.	Pill-9mm.								
9062	2	Mag. Al.	Pill-7mm.	==	==	==	==	==	==			
8886	2	Mag. Ars.	Pill-9mm.	==	==	==					
8947	2	Mag. Blues.	Pill-1 cm.	==	==	==	==	==			
9612	2	Ars. Mag. Blues.	Pill-9mm.	==	==	==	==				
8325	2	Ars. Cooper's-	Powder	==	==	==	==	==				
Lamb 4 months	2	Blues. plus Mag.	Powder	==	==	==	==	==				
	2	Mag.	Pill-9mm.	==	==	==						
10676	2	Mag.	Powder	==	==						
8511	3	Fat and Sudan	A Tea- spoon	==	==	==						
8133	3	Al.	Powder	==	==	==				
10013	3	Mag.	"	==	==	==	==	==	==		
7717	3	Mag. Al.	Pill-mas- ticated	==	==	==	==	==	==			
9694	3	Mag. Blues.	Pill-9mm.	==	==	==	==				
9691	3	Ars. Mag. Blues.	"	==	==	==	==	==			
8133	4	Ars. Cooper's-	Powder	==	==	==	==	==			
9731	4	Blues. Mag.	Pill-9mm.	==	==	==	==	==				
9715	4	Ars. Mag. Blues.	"	==	==	==	==	==			
10731	4	Ars. Mag. Al.	Powder	==	==	==	==	==	==			
9238	5	Al. Mag.	"	==	==	==	==	==				
9728	5	Mag. Blues.	Pill-9mm.	==	==	==	==	==			
10729	5	Ars. Al. Mag.	Powder	==	==	==	==	
9427	10	Al.	"	==	==	==	==	==				
9427	10¼	Mag.	"	==	==	==	==	==				
9725	24	Blues. Ars.	"	==	==	==	==	==			
9591	24	Mag.	"	?	?	?	?	? Droppings		stained	In pink.	
9582	24	"	"	?	?	?	?	?	Faeces	"	"	red.
8969	24	Blues. Ars.	"	==	==	==	==	==			?	
		Mag.	"	==	==	==	==	==				

No. of Sheep.	Time after Dosing.	Substance Administered.	Form of the Substance.	Ru-men.		Reticulum	Omasum.	Aboma-um.	Jejunum.	Ileum.	Large Intes-tine.	
				1	2						3	4
9434	hours. 24	Mag. Al.	Powder	=====	=====	=====	=====	=====	=====	=====	=====	=====
9416	24	"	"	=====	=====	=====	=====	=====	=====	=====	=====	=====
9416	single dose 24	"	"	?	?	?	?	?	Faeces	stained	in	red.
9591	double dose 48	"	"	=====	=====	=====	=====	=====	=====	=====	=====	=====
10641	double dose 48	Blues. Ars	"	=====	=====	=====	=====	=====	=====	=====	=====	=====
10763	single dose 3 days	Mag. Blues. Ars.	"	=====	=====	=====	=====	=====	=====	=====	=====	=====
9582	double dose 4 days	Mag. Al.	"	=====	=====	=====	=====	=====	=====	=====	=====	=====
9519	double dose 9 days	"	"	=====	=====	=====	=====	=====	=====	=====	=====	=====
	single dose			=====	=====	=====	=====	=====	=====	=====	=====	=====

Explanation of the above Table.

1. The following abbreviations were used:—Al.=aluminium powder; mag.=magenta stain; blues.=bluestone (copper sulphate); ars. = sodium arsenite.

2. The lengths of the horizontal lines crossing the columns indicate how far the stain was detected in the alimentary canal. When the line is discontinued it indicates that the stain was not noticed in the intestinal portion indicated at the head of the column. This refers to the rumen, where the stain was only noticed in the anterior dorsal blind sac and not in the remaining part of the same diverticulum.

3. _____ A plain single line indicates the portions of the digestive tracts in which the stain was homogeneously red.

===== The double line indicates when the staining was very marked.

..... The dotted line indicates only traces of stain.

4. For the column "Rumen" the number "1" indicates the "anterior superior blind sac of the rumen"; the number "2" indicates the rest of the rumen. In the column "large intestine" the number "3" indicates the "cæcum and great colon"; the number "4" indicates the "small colon and rectum."

5. In the column "Time after dosing," the sign "o" indicates that the sheep was kept on its haunches during dosing, and the sign "||" indicates that the sheep was kept standing.

6. The "question mark" in the results of numbers 9591, 9582, 9416, means that the contents of the digestive canal were not examined, as the sheep were not killed.

(c) PASSAGE OF COLOURING AGENTS IN THE STOMACHS OF SHEEP WHEN TAKEN VOLUNTARILY.

(1) *Colouring Agents licked by the Sheep.*

In twelve cases sheep were allowed to lick voluntarily sodium chloride mixed with magenta stain for about 3-5 minutes and were

killed afterwards. In ten cases sheep were killed after 5 minutes—Nos. 9451, 9513, 11360, 10828, 11095, 10803, 10944, 11017, 11346 (*vide* table).

On post-mortem the mucosa of the mouth and of the pharynx was dark red; the mucosa of the oesophagus pink in colour. The contents of the anterior superior blind sac of the rumen were stained red; patches of red were found on the periphery of the rumenal contents. The omasum was completely free from red stain and so was the abomasum. The contents of the rumen were of average size and consistence in all the sheep, with the exception of one case in which the consistence was semi-fluid. In this last case (No. 11323 in the following table) the contents of the rumen and reticulum were homogeneously tinged with pink, but no traces of stain were detected in the omasum and abomasum. In one case (No. 10912, *vide* table) the sheep was killed one hour after licking the salt and magenta. The sheep did not ruminate after the lick. At post-mortem the contents of the rumen and reticulum were found rather solid and homogeneously stained in red; the sulcus omasi and the upper portion of the leaves of the omasum were red in colour. The abomasal contents were fluid and of a pink colour. No traces of stain were present in the small intestine. In all the cases reported the sheep were kept in the stable and induced to lick the salt about three to four hours after the morning meal (7 a.m.).

(2) *Colouring Agents drunk by Sheep.*

In six cases sheep were kept without water for 12 to 24 hours, then offered water stained with magenta, and killed 5 to 10 minutes after drinking. In three cases out of six (Nos. 10426, 9446, 11400) the contents of the anterior superior blind sac of the rumen and of the reticulum were found intensely red, and the contents of the rest of the rumen fairly red. In another case (No. 9466) the contents of the rumen and reticulum were homogeneously red. In another case (No. 11176) the greater part of the drug was in the anterior superior blind sac and only traces were present in the reticulum. In another case (No. 11376) the colouring stuff was present only in the rumen, with nothing in the reticulum. In the last two cases (Nos. 11176 and 11376) the contents of the rumen were remarkably dry, and it appears that no water fell in the reticulum. In none of the above cases was it possible to detect traces of red in the omasum and abomasum.




















































Remarks.

1. In the following table the same explanation holds about the conventional words as used in the previous table, with the exception that in column "Time after dosing" the letter "l" is used to indicate that the sheep licked the powder and the letter "d" indicates that the sheep drank the liquid voluntarily.

2. No. 11376 drank only a few mouthfuls, and as the rumen content was rather dry the water was at once absorbed by the contents of the anterior superior blind sac.

3. No. 11176 drank about 400 c.c. of stained water. No. 9644 drank for 2-3 minutes, and a lot of blue liquid was found in the rumen and reticulum.

(3) *Graphic Demonstration of the Passage of Drugs in the Alimentary Canal of the Sheep when taken Voluntarily.*

No. of Sheep.	Time after Dcsing.	Substance Administered.	Form of the Substance.	Ru-men.		Reticulum.	Omasum.	Abomasum.	Jejunum.	Ileum.	Large Intes-tine.	
				1	2						3	4
	minutes											
9451	5 l.	Mag. Salt.	Powder									
9513	5 l.	"	"									
11360	5 l.	"	"									
10828	5 l.	"	"									
11095	5 l.	"	"									
10803	5 l.	"	"									
10954	5 l.	"	"									
11017	5 l.	"	"									
11346	5 l.	"	"									
11323	5 l.	"	"									
11122	15 l.	"	"									
10426	5 d.	"	Solution									
9446	5 d.	"	"									
11376	5 d.	"	"									
11176	10 d.	"	"									
9466	5 d.	"	"									
11400	5 d.	"	"									

Conclusions of the Passage of Colouring Agents when taken Voluntarily.

1. It appears that a liquid, or soluble solid, taken voluntarily by the sheep is invariably passed to the rumen and reticulum.

2. In case of soluble powder the greater part of the ingested material is passed to the anterior dorsal blind sac. A fair amount is also passed to the reticulum, whether by simple diffusion or by the mechanism of the rumen-reticular apparatus could not be said.

3. In the case of liquid substance it is passed by deglutition on to the contents of the anterior dorsal blind sac of the rumen, from where it falls in considerable amount into the reticulum. Where the contents of the rumen were rather dry the water has mostly been absorbed by the ingesta of the anterior dorsal blind sac (No. 11176).

4. In 10-15 minutes the dissolved material, or liquid ingested, is mixed up with the rumenal contents. After this time the passage of the drug is presumably the same as in the case of substances administered forcibly to the sheep.

5. It is to be specially remarked that substances licked or swallowed voluntarily by the sheep never passed, not even in fraction, to the abomasum, in any of the seventeen cases observed.

(f) PASSAGE OF ADMINISTERED DRUGS THROUGH THE ALIMENTARY CANAL
OF THE SHEEP AS SHOWN BY CHEMICAL ANALYSIS.

The substances used in the following series of experiments were sodium arsenite and bluestone mixed in definite proportions on the basis of the arsenical equivalent, which is reported in the following table. Various modes of dietetic treatment were also applied. At the prearranged time after dosing, the sheep were killed, and the requisite material taken by Dr. Green, to whom the writer is indebted for the following table, taken from his own paper upon the fate of ingested arsenic.

Sheep No.	Dietetic Treatment.	Hours between Dosing and Death.	Arsenic Equivalent of the Dose of As_2O_3 .		Rumen.		Reticulum.		Omasum.		Abomasum.		Total in Stomachs expressed as per cent. of the Dose Administered.	Small Intestine.		Large Intestine.	
			mg.	g.	mg.	g.	mg.	g.	mg.	g.	mg.	g.		g.	mg.	Weight of As_2O_3 Content.	Weight of As_2O_3 Content.
8325	{ S 17..... F..... }	2	105	2,350	15	65	2	55	14	260	54	81	Nearly empty	Trace	520	2	
8133	{ S 17..... F..... }	4	105	2,000	6	150	4.5	45	4.5	150	26	39	190	3	570	2	
10676	{ S 17..... F..... }	5	120	2,740	14	120	4.5	—	15	820	36	58	350	1.5	675	Trace	
10868	{ S 17..... F..... }	8	100	1,870	15	53	0.7	24	1.6	525	16	33	—	—	—	—	
10576	{ S 17..... F 5..... }	8	100	2,540	43	55	0.6	18	1	135	3	48	250	1	550	Trace	
10680	{ S 17..... F 5..... }	8	100	1,480	7	91	1	38	2.5	255	30	41	250	1	775	Trace	
9705	{ S 17..... F 3-6 (water only) }	8	100	4,600	62	98	1.8	87	2.7	30	Trace	67	—	—	—	—	

Sheep No.	Dietetic Treatment.	Hours between Dosing and Death.	Arsenic Equivalent of the Dose As_2O_3 .		Rumen.		Reticulum.		Omasum.		Abomasum.		Total in Stomachs expressed as per cent. of the Dose Adminis-tered.	Small Intestine.		Large Intestine.	
			mg.	g.	mg.	g.	mg.	g.	mg.	g.	mg.	g.		Weight of As_2O_3 Content.	g.	Weight of As_2O_3 Content.	g.
11362	{ S F (not discontinued, but not water)	8	100	5,020	62	20	Trace	31	0.5	340	2	65	—	—	—	—	—
11357	{ S 17 F 5	8	100	4,220	69	387	7	68	1	375	3	80	—	—	—	—	—
10638	{ S 17 F}	12	120	3,320	15	118	3	50	4.5	200	4	22	400	1.5	480	1.5	1.5
8969	{ S 17 F 5	24	105	2,800	13	90	7	70	2	300	7	28	680	3.5	600	12	12
9725	{ S 17 F 5 (very little eaten)	24	120	1,550	26	100	2	Total arsenic, 5 mg.		Trace		28	400	3	17	Trace	Trace
10641	{ S 17 (1) F 5	48	120	1,500	1	95	Trace	—	Trace	—	Trace	1	—	Trace	500	1.5	1.5
10753	{ S 17 (2) F 5	12	120	2,750	27	130	1.3	60	4	150	Trace	27	390	1.5	550	14	14
10763	{ S 17 (3) F 5	72	120	3,460	2	200	Trace	95	Trace	275	Trace	2	330	Trace	530	4	4

Remarks on the above Table.

(1) It will be noticed that some of the sheep reported have already been mentioned in the table dealing with the colouring substances. The reason is that some sheep were dosed at the same time with arsenite, bluestone, and magenta. At the post-mortem the fate of the colouring substances was observed while the sampling manipulations for chemical analyses were proceeding. (2) The sheep used in the above experiments were all in fairly good condition. No. 10576 was 2-tooth, and No. 8969 was 4-tooth; all the other sheep were full mouth. (3) During treatment sheep were kept in a stable enclosed in a loose box, the temperature being mild or warm on the days of the experiments. (4) Sheep were kept seated on their haunches during the administration of the dose, with the exception of No. 11387 (injected through the flank), which was kept standing on its four legs. (5) Green grass, usually lucerne mixed with a small amount of hay, was used when sheep were fed during the time that elapsed from the dose to the time of death. (6) The arsenic was administered in Nos. 8325, 8133, 8969, in the form of Cooper's dip powder, and in all the other cases as commercial arsenite of soda. (7) In every case of dosing reported in the above table, the arsenical dose was mixed with 500 mg. of bluestone. The bluestone was added for the purpose of producing on the function of the alimentary canal the same effect as in the dosing which will be recommended in the present paper. The mixture was in powder form, with the exception of No. 11387, in which the dose was liquid, and injected directly into the rumen. (8) In the column under "Dietetic treatment," "S" is used to signify that the sheep was starved, i.e. kept without food and water for the number of hours expressed by the side figure. "F" in the same column indicates that the sheep was fed but not watered after dosing, the interval in hours being expressed by the side figure. When no side figure is indicated it means that sheep did not receive food after the dose. (9) The detection of arsenic eight hours after dosing was taken into special consideration, because it gives the fate of arsenic as a result of the first meal. Thus, No. 10868 gives the fate of arsenic when the first meal is omitted or postponed; No. 10576 when the first meal is taken properly; No. 10680 was a control of the previous sheep, taking into consideration the different age; No. 9705 is intended to show the effect of water ingested after the dose; No. 11362 shows the effect of food previously and after dosing; No. 11387 shows the fate of arsenic when the dose is quantitatively mixed with the contents of the rumen. (10) No. 10641 had green food five hours after dosing, was starved again until the following morning, then fed and offered water as usual. This sheep showed diarrhoea on the second day of treatment. Nos. 10753 and 10763 had the double dose treatment, viz., dosed on two successive days, and were kept without food and water for seventeen hours previously, and five hours after each dose.

Conclusions on the Passage of Arsenic through the Alimentary Canal.

(1) Two hours after dosing, 50 per cent. of the arsenic was found in the abomasum. Apparently half the dose given remained on the

anterior dorsal surface of the rumenal content, from where it was brought in the stream of the rumenal revolutions; the other half of the dose presumably fell in the reticulum from where, as it was said with regard to the staining substance, it was passed to the abomasum. It is a remarkable fact that in two hours traces of arsenic were found in the large intestine. When colouring agents were used, staining of the contents was not observed so soon. This might be explained by the more delicate finding of chemical analysis. (2) After four and five hours the main part of arsenic is passed to and through the abomasum. Arsenic is detected up to the large intestine. (3) Eight hours after the dose the following results were obtained: In the case where the sheep did not feed (No. 10868), equal amounts of arsenic were found in the rumen and in the abomasum. It appears that the part of arsenic passed into the rumen was still carried in the rumenal revolutions, and the part that passed to the reticulum was already poured into the abomasum. With No. 10576, which had food five hours after the dose, the amount of arsenic passed to the rumen was still there, and the part passed into the reticulum, which at the time of feeding was presumably in the abomasum, was by the increased digestive activity after the meal, passed further, or absorbed. In a second case, under the same conditions (No. 10680), the dose was passed for the main part into the reticulum, and at the eighth hour the dose was partly in the abomasum and partly absorbed. In the sheep which had water after dosing (No. 9705) it appears that a very considerable part of arsenic fell into the rumen and remained there, but that the water was passed on and quickly washed out the arsenic from the abomasum. Also in the case when feeding was not discontinued during the dosing (No. 11362) the small amount of arsenic present in the abomasum was quite remarkable. Both experiments tend to show that food and water after dosing hasten the passage of the reticular arsenic content through the abomasum with resulting diminution of effect on the parasites. In the case where the arsenic was injected into the rumen, it appears that the dose was passed gradually and in high dilution into the abomasum. This differs from the majority of the other observations made by the administration of the dose through the mouth, where a primary flow of rather concentrated arsenic solution from the reticulum to the abomasum was noticed, followed by a slow, very diluted flowing of arsenic from the rumen. The difference appears to be of high importance in the effect of treatment on the wire-worms. (4) After twelve hours (No. 10638) most of the arsenic that had passed into the reticulum as a result of ingestion is already in the abomasum and intestines and absorbed in the system, while fractions have penetrated into the large intestine. A considerable proportion of the arsenic that had passed to the rumen as a result of deglutition was detected further along the digestive canal up to the small intestines. (5) Twenty-four hours after the ingestion (Nos. 8969 and 9725) the arsenic that fell in the rumen is still found to pass slowly from the rumen and to proceed further in the digestive tract; a fair amount of arsenic is sometimes present in the large intestine. It is worth noting how the meal five hours after the dose pushed the arsenic away from the rumen in comparison with the following number (No. 10641) that practically did not eat, and in which the arsenic was allowed to stagnate longer in the rumen as a result of the low activity of the same reservoir. (6) Forty-eight hours after the injection (No. 10641): Only traces of arsenic can be found

in the alimentary tract. The only places where it can be detected in appreciable amount are those in which the ingesta lodge, i.e. the rumen and the large intestine. In the canal between these two reservoirs, where the ingesta are flowing rather fast, and the walls are particularly absorbent, the arsenic is in trifling amount. It may be mentioned here, in the case of sheep No. 10641, in the first twenty-four hours after the dose, 1.5 per cent. of the arsenic was passed in the faeces, and 34 per cent. was passed in the urine, and that in the second day 21 per cent. of the given arsenic was passed in the urine. (For further details concerning elimination of arsenic, Dr. Green's own paper may be consulted.) In comparing these figures with those arrived at with sheep killed twenty-four hours after the dose, it appears that the feeding of the second day must be chiefly responsible for the washing out of the arsenic. This fact shows once more that the period of starvation which will be suggested in the treatment of sheep for wire-worm is really indispensable, allowing the arsenic to remain longer in the various portions of the alimentary canal, and thus more in contact with the parasites than it would if the feeding was not discontinued. (7) Twelve hours after the double dose the same feature in the distribution of arsenic is noticed as for twelve hours after the single dose, but the arsenic is in the rumen in a higher amount. If the sheep shows diarrhoea, the arsenic can be present in the colon in considerable amount. Forty-eight hours after the double dose the distribution of arsenic is as said for twelve hours, but the amount of arsenic in the alimentary canal is considerably reduced. (8) From the above table it results that the amount of sodium arsenite in the intestine is in general very small owing to its easy absorption through the walls of the abomasum and jejunum. In cases in which there is diarrhoea the arsenic can be found in considerable amount in the large intestine, owing to the decreased absorption and the increased peristalsis of the alimentary canal.

General Conclusions on the Experiments dealing with the Fate of the Drugs in the Digestive Tract of a Sheep.

The results of the experiments on the fate of the colouring substances show a fair uniformity and connection, but it is more difficult to draw general definite conclusions when experiments with colouring substances and arsenic are compared. This is probably attributable to the higher diffusibility of sodium arsenite and its quicker absorption through the mucosa and on the more perfect method of detection adopted for the arsenic than for the colouring substances. Notwithstanding, the following conclusions appear justified:—*First thirty minutes after injection*: Passage of the drug as a result of the deglutition. *Liquid form*: Liquids administered to a sheep in a quantity from 10 to 140 c.c. usually pass "in bulk" to the anterior dorsal blind sac of the rumen, and to the reticulum (86 per cent. of the cases observed). Liquids swallowed voluntarily by a sheep always pass to the anterior blind sac of the rumen and to the reticulum. These two statements imply the view that the passage of liquid drugs directly from the oesophagus to the abomasum, either in fractions or "in bulk" (14 per cent. of the recorded cases) is an accidental deviation from the normal stream of ingested fluid, as a result of the forced deglutition of the

substance. *Powder form*: A soluble powder is frequently moistened in the mouth and passed undissolved to the anterior dorsal blind sac of the rumen, and to the reticulum, or in proximity to the reticulo-omasal orifice. This happens chiefly when sheep are considerably excited on account of the forced administration of the dose. At other times the powder is dissolved in the mouth and the solution in saliva is passed in the way described for the "liquid form." In dosing, this happens in the majority of cases, and it is invariably the case when the powder is licked voluntarily by the sheep. *Pill form*: When the pill is masticated there is no appreciable departure from the deglutition of the powder form. When the pill is swallowed without being masticated it falls in the anterior blind sac of the rumen or in the reticulum. In the cases recorded in the present paper the pill was never found directly in the abomasum, even when pills of 5 mm. diameter were administered. Not seldom the pill is passed into the pharynx, retained for a considerable length of time and then spat out. The ingested pill, if made with acacia gum, is generally dissolved in 15-20 minutes.

Fate of the Drug immediately after Ingestion: When the rumenal content is normal in volume (from 2000 c.c.) and in consistency (semi-solid) the revolutions of the rumen draw clumps of ingesta embedded with portions of drug from the anterior dorsal blind sac, and bring them near the walls of the rumen along the dorsal curvature, the posterior dorsal blind sac, the ventral curvature of the rumen, and finally mix them with the central portion of the rumen content. When the rumenal contents are scanty (under 1500 c.c.) and fluid, the drug is sometimes mixed immediately with the reticulum and rumen contents (the anterior dorsal blind sac being collapsed or distended by gas) and sometimes the solid drug falls in the reticulum only, where it remains for hours. One hour later the substance, whether given in liquid, in powder, or in pill form, is now mixed in lumps with the rumen content, or homogeneously mixed with the entire bulk. The sulcus omasi is usually also stained, and it happens sometimes that portions of the given substance are already present in the abomasum, but near to the omaso-abomasal orifice. *One and a half to two hours*: After one and a half hours the drug may have reached the abomasum in fair amount. After two hours (50 per cent. of the recorded cases) the drug is homogeneously mixed from the rumen to the abomasum. In few cases the solution is more intense in the abomasum than in the rumen, and may be as high as 50 per cent. of the dose given. In a few instances the substance given is present in traces or in fair amount in the jejunum (magenta) and large intestine (arsenic). *Three hours*: After three hours the solution of the drug has frequently reached the jejunum (50 per cent. of cases recorded), and one case was recorded in which traces of the drug were present in the large colon. *Four hours*: After four hours the colouring substance was always found homogeneously diluted in the four divisions of the stomach, and in the majority of the cases also present in the jejunum (magenta). Arsenic was found in the large intestine. *Five hours*: After five hours the drug is diluted in the alimentary canal from the rumen to the ileum, and frequently traces are present in the large intestine (magenta-arsenic). *Eight to ten hours*: In the experiments with the colouring agents in which the sheep were kept without food or water,

the dose was found from the rumen to the abomasum with the higher concentration in the reticulum. Drugs not easily absorbed can be present in the large intestine in a fair amount. In the experiments with arsenic (eight hours) the dose was found more than half in the rumen in 50 per cent. of the cases, nearly half in 60 per cent. of the cases, and in 34 per cent. of the instances the bulk of the dose was in the abomasum or absorbed into the system. *Twelve hours:* The part of the drug that was in the reticulum has already gone through the abomasum, and the part that was passed to the rumen is slowly pushed further in the alimentary tract in a weak solution. The abomasum then contains a feeble, fairly constant solution of drug (arsenic). In cases in which arsenic is not mostly absorbed in the small intestine (diarrhœa) the said drug can be present in the large intestine in considerable proportion. *Twenty-four hours:* A considerable amount of drug is still stored in the rumen and reticulum (compare both tables with colouring substances and with arsenic). The remaining part of the digestive canal contains throughout a weak solution of the drug. Part of the substance administered is in the system (arsenic), and part has already been passed out with the faeces (magenta-arsenic) and with the urine (arsenic). *Forty-eight hours:* From both tables of staining substances and arsenic it results that, when in the first day the starvation system, and in the second day the normal dietetic system, is applied, some amount of drug can still be detected in the rumen and colon, and in the remaining alimentary tract only traces of the drug are found.

Double dose.—Twelve hours: The drug (arsenic) is distributed in the alimentary canal very similarly to twelve hours after the single dose, with the difference that the amount of arsenic in the rumen or large intestine is higher. *Forty-eight hours:* The same distribution of drug is found as said for the single dose, with the difference that the amount of drug in the rumen is noticeably higher (compare No. 9591 with No. 10641 in the table of colouring substances). *Three days:* The higher concentration of drug is still detected in the rumen and colon, although the figures are very small. Only traces of drug are detected in the abomasum and small intestine (arsenic). When the function of the abomasum and intestines is not disturbed to a great extent, the easily absorbed drugs (sodium arsenite) are passed into the system for the most part through the mucosa of the abomasum and jejunum. It is only in cases of abnormal stimulation of the alimentary canal, resulting usually in diarrhœa, that a continuous amount can be passed to the large intestine. From this fact it can be concluded that when a vermicidal effect is wanted in the large intestine by use of an easily absorbed arsenical compound, the drugs administered through the mouth must act as purgative on the stomach and small intestine. As a general conclusion applied to the treatment of wire-worms, that will be suggested further in the present paper, it appears that worms in the abomasum are submitted to acute poisoning action for the first five to eight hours after the dose, and to a slow constant poisoning action for at least three days after the second dose.

B.—ACTION OF DRUGS ON *HAEMONCHUS CONTORTUS* “IN VITRO.”

INTRODUCTION.

With the object of studying the larvae in a condition resembling as near as possible that under which they are present in the abomasum, the following points were taken into special consideration:—

(a) *Viscosity of the medium in which the larvae were observed*: A saturated solution of acacia gum was used, which was found to be harmless to the larvae and having approximately the viscosity of the mucus coating the abomasal walls of a sheep. (b) *Acidity of the medium*: To the solution of acacia, 0.3 per cent. of hydrochloric acid was added so as to give to the medium the acidity usually found in the abomasum. (c) *Temperature and light*: The fluid containing the larvae under observation was kept in the incubator at 37°-38° C. (d) *Terms used in the tables dealing with the resistance of larvae and adult worms to the action of drugs “in vitro”*: For the uniform and methodical determination of the appearance of the parasites when under observation, the following definitions are given to the various words used:—*Swimming* (swim.): The larvae were observed to have the same movements as when they were immersed in pure water. *Moving* (mov.): The larvae simply twisted themselves about more or less actively without travelling to another place. *Reacting* (reac.): The larvae were motionless from the instant when taken out of the incubator, but showed some movements from time to time when exposed to either action of light or to a different temperature, or when subjected to any mechanical motion. *Motionless* (mot.): The larvae did not react under the conditions referred to in the heading “reacting,” but yet did not present the straight or curved appearance typical for dead larvae. Under these conditions the larvae would be considered as being either in a state just previous to or immediately after death. *Dead*: The larvae presented the typical straight or curved appearance associated with death. Bearing in mind the warning of Looss, in many instances the death of larvae or adult worms was verified by immersing them in pure physiological water for 24 hours at a temperature of 38° C. In each of the following tables the observations bearing the same numerical index were made at the same time, in the same incubator, and either with the same culture of larvae or with adult worms from the same host. It was intended to use *coal-tar creosote* in place of *beech-tar creosote* for the sake of closer comparison with the experiments carried out by Ransom in America, but at the time these experiments were undertaken coal-tar creosote was not available in South Africa.

(e) ACTION OF DRUGS ON “*HAEMONCHUS CONTORTUS*” AT THE LARVAL STAGE: MATURE LARVAE.

(1) *Resistance of Larvae in Saturated Solutions*: The following observations were made at the time when dosing experiments with bluestone, Cooper’s dip, and some arsenical compounds were being carried out in sheep, and it was interesting to see the effect of the same drug on the larvae and adult *Haemonchus contortus* “in vitro.” The results of the observations are summarized in the following table:—

Drugs Used in a Saturated Solution.

Drug.	Index No.	Time of Observation.						
		15 Mins.	1 Hour.	5 Hours.	10 Hours.	17 Hours.	24 Hours.	48 Hours.
Sulphate of copper.....	1	Swim. —	80 mov. 20 dead	30 mov. 70 dead	— —	— 11 dead	—	—
	2	50 swim. 50 mov.	90 mov. 10 dead	30 mov. 70 dead	1 mov. 99 dead	— —	— All dead	—
Cooper's dip.....	1	Swim. —	50 swim. 50 mov.	85 mov. 15 dead	— 25 dead	— —	— 40 reac. 60 dead	1 reac. 99 dead
	2	50 swim. 50 mov.	85 mov. 15 dead	80 mov. 20 dead	65 mov. 35 dead	— —	20 reac. 80 dead	1 mov. 99 dead
Sodium arsenite.....	1	75 mov. 25 dead	33 mov. 66 dead	(2 hours) All dead	(3 hours) All dead			
	2	50 mov. 50 dead	50 mov. 50 dead	(2 hours) 90 dead				
	3	80 mov. 20 dead	60 mov. 40 dead	(2 hours) 99 dead				
Arsenious oxide.....	1	Swim. —	99 swim. 1 reac.	95 mov. 5 dead	— —	50 reac. 50 dead	20 reac. 80 dead	All dead
	2	Swim. —	90 swim. 10 reac.	90 mov. 10 dead	— —	— —	10 reac. 90 dead	— All dead
1 part Cooper's dip, 4 parts sulphate of copper	1	40 swim. 60 mov.	90 mov. 10 dead	0.5 reac. 99.5 dead				
	2	50 swim. 50 mov.	95 Mov. 5 dead	0.5 reac. 99.5 dead				

Conclusions.

1. A concentrated solution of sodium arsenite proved to be the most poisonous of the drugs used, and was followed by Cooper's dip and sulphate of copper, arsenious oxide, Cooper's dip.

(2) *Resistance of Larvae in 0.5 per cent. Solutions.*—In testing the resistance of larvae, saturated solutions are of limited value, as the drugs are not present in the same proportion in the solution, but only in proportion to their solubility. For the subsequent experiments the proportion of 0.5 per cent. was used in which the drugs used were completely dissolved. It was also observed that with some drugs in a 0.5 per cent. solution, the larvae survived for a considerable time, thus allowing more accurate observations. The drugs previously used in saturated solution were tested again in a 0.5 per cent. solution and the results were compared with drugs, generally considered as possessing anthelmintic properties. The observations are recorded in the following table:—

Drugs Used in a 0.5 Per Cent. Solution.

Drug.	Index No.	Time of Observation.									
		15 Mins.	1 Hour.	2 Hours.	5 Hours.	24 Hours.	2 Days.	3 Days.	4 Days.	5 Days.	7 Days.
Sodium arsenite.....	1	99 mov. 1 dead	98 mov. 2 dead	98 mov. 2 dead	75 mov. 25 dead	15 reac. 85 dead	5 reac. 95 dead	— All dead	—	—	—
Sulphate of copper.....	1	— Swin.	Swin. —	99 swim. 1 dead	99 swim. 1 dead	90 mov. 10 dead	30 mov. 70 dead	30 mov. 70 dead	30 mov. 70 dead	25 mov. 75 dead	30 mov. 70 dead
Cooper's dip.....	1	Swin.	98 swim. 2 dead	98 swim. 2 dead	90 swim. 10 dead	75 swim. 25 dead	66 mov. 33 dead	50 mov. 50 dead	30 mov. 70 dead	(6 days) 90 dead	(8 days) 97 dead
Lysol.....	1	95 swim. 5 dead	50 mov. 50 dead	20 mov. 80 dead	(3 hours) All dead						
Creosote (beech-tar).....	1	5 reac. 95 dead	(20 mins.) 99 dead	(30 mins.) 99 dead	(35 mins.) All dead						
	2	5 reac. 95 dead	(20 mins.) 99 dead	(30 mins.) All dead							
	3	5 reac. 95 dead	(20 mins.) 99 dead	(30 mins.) All dead							
Gasoline.....	1	99 swim. 1 dead	99 swim. 1 dead	— —	— —	95 swim. 5 dead	80 swim. 20 dead				
Acacia saturated solution (control)	1	— —	— —	99 swim. 1 dead	— —	98 swim. 2 dead	98 swim. 2 dead	— —	— —	95 swim. 5 dead	80 swim. 20 dead

Observations.

Creosote proved to be by far the most effective, with lysol as a poor second. Sodium arsenite came next, followed by sulphate of copper and Cooper's dip. Gasoline was the least effective. In comparing sulphate of copper with Cooper's dip there is the striking fact that until the third day sulphate of copper was more active; from the fourth day onwards Cooper's dip surpassed sulphate of copper, and although not stated in the above table, even on the twelfth day 0.1 per cent. of larvae were still reacting in the Cooper's dip solution. Observations on the larvae in gasoline solution were discontinued from the second day, as it appeared that the solution was exhausted and the larvae behaved similar to that occurring with pure acacia solution.

(f) ACTION OF DRUGS ON "HAEMONCHUS CONTORTUS" AT THE
ADULT STAGE.

In order to have suitable material for the required observations, sheep were heavily infected with larvae of *Haemonchus contortus*, and were killed when the clinical symptoms and cultures made from faeces allowed the diagnosis of heavy infection. The abomasum was taken out with its contents and the worms transferred at once to various solutions.

(1) *Resistance of Adults in 0.5 Per Cent. Solution.*

Drug.	Index No.	Time of Observation in Minutes.										
		5.	10.	15.	20.	25.	30.	40.	50.	60.	70.	80.
Sodium arsenite.....	1	Swim. — Swim.	Swim. — Swim.	Swim. — Mov.	Mov. 50 mov. 50 reac.	Mov. 50 mov. 50 reac.	Reac. 50 reac. 50 dead	90 reac. 10 dead Dead	50 reac. 50 dead	10 reac. 90 dead	Dead	
Sulphate of copper.....	1	—	—	—	—	—	—	(4 hours) Swim.	(5 hours) 70 mov. 30 dead	(7 hours) 60 mov. 40 dead	(10 hours) 30 reac. 70 dead	(22 hours) All dead
Sulphate of copper.....	1	Swim.	Swim.	Swim.	Swim.	Swim.	90 swim.	50 swim.	90 mov.	90 mov.	10 reac.	—
Sodium arsenite.....		—	—	—	—	—	10 mov.	50 mov.	10 dead	10 dead	90 dead	Dead
Creosote.....	1	Swim. — Swim.	Swim. — Swim.	Swim. — Swim.	Swim. — Swim.	80 swim. 20 mov.	50 mov. 50 reac.	50 mov. 50 dead	40 reac. 60 dead	10 reac. 90 dead	Dead	—
Lysol.....	1	Swim. — Swim.	60 swim. 40 mov.	— —	Mov. —	— —	50 mov. 50 reac.	75 reac. 25 dead	60 reac. 40 dead	20 reac. 80 dead	10 reac. 90 dead	Dead
Thymol.....	1	Mov. 50 mov. 50 reac.	Reac. Dead	Dead	80 mov. 20 reac.	— —	Reac. —	50 reac. 50 dead	20 reac. 80 dead	5 reac. 95 dead	Dead	
Beta-naphthol.....	1	Mov.	80 mov.	Reac.	20 reac.	Dead	—	—	—	—	—	
Acacia saturated solution.	1	Mov.	20 reac.	Dead	80 dead	—	—	—	—	(5 hours) All alive	(10 hours) 75 mov. 25 mov.	(22 hours) Dead

Time of Observation, in Minutes.

Drug.	Index No.	Time of Observation, in Minutes.										
		5.	10.	15.	20.	25.	30.	40.	50.	60.	70.	80.
Sodium arsenite.....	2	Swim. Swim.	Swim. Swim.	Mov. Mov.	Mov. 80 mov. 20 mot.	Mov. 50 mov. 50 mot.	Reac. 25 reac. 75 dead	75 reac. 25 dead Dead	20 reac. 80 dead	Dead		
Cresote.....	2	Mov. —	50 mov. 50 dead	Dead								
Thymol.....	2	Mov.	Dead									
Beta-naphthol....	2	Mov. —	50 reac. 50 dead	25 reac. 75 dead	(17 mins.) Dead							
Picric acid.....	2	Swim. — —	50 swim. 50 mov. —	50 swim. 50 mov. —	50 swim. 40 mov. 10 dead	— — —	50 mov. 25 reac. 25 dead	10 mov. 65 reac. 25 dead	60 reac. 40 dead	10 reac. 90 dead	Dead	
Acacia saturated solution.	2	— —	— —	— —	— —	— —	— —	— —	— —	(5 hours) All alive	(11 hours) 80 mov. 20 dead	(22 hours) Dead
Sodium arsenite (tempera- ture, 30° C.)	3	Swim. —	Swim. —	25 swim. 75 mov.	Mov. —	Mov. —	50 mov. 50 reac.	95 reac. 5 dead	50 reac. 50 dead	25 reac. 75 dead	5 reac. 95 dead	Dead
Cresote.....	3	50 swim. 50 mov.	50 mov. 50 reac.	10 reac. 90 dead	Dead							
Thymol.....	3	50 mov. 50 reac.	Dead									
Beta-naphthol.....	3	50 mov. 50 reac.	50 reac. 50 dead	Dead								

Remarks.

(1) Studying the effect of drugs on worms of different ages a sheep was used that was naturally infected with wire-worm, and this sheep was then further artificially infected with larvae. Nineteen days after infection the sheep was killed, and the adult worms of both infections were transferred to the medium. As the two kinds behaved differently under the action of the drugs, the behaviour of the worms surviving from natural infection has been underlined in the table. It was, however, not possible to detect difference in the experiments with bluestone and with saturated solution of acacia, owing apparently to the drug being too weak. (2) In the second experiment young worms which had just reached the stage of copulation were used. A few adult full-grown worms found in the abomasum mixed with the lot of young worms, were transferred into the solution of sodium arsenite, and the result of their resistance is underlined. (3) In Experiment No. 3, the worms in sodium arsenite were left at room temperature which at the time was 30° C.

Conclusions.

(1) In comparing the three series of experiments in the above table, it appeared that strains of worms from different hosts show almost the same sensibility to the same drug. (2) In comparing the batch of old worms with the batch of young worms in the first experiments, and in comparing the second with the third experiment, it appeared that young worms were less resistant to the same drug than the full-grown worms. (3) The fact that the worms in the third experiment were killed by sodium arsenite (kept at 30° C.) 10 minutes later than in the first experiment and 20 minutes later than in the second experiment, tends to show that lower temperatures weaken the action of the drug, but the results in the first and second experiments were not constant enough to state this definitely. (4) Thymol proved to be the most poisonous of the drugs used in the above table. It killed the full-grown worms in 10-15 minutes and the adult young worms in 10 minutes. (5) Creosote and beta-naphthol were almost on a par and acted 5-10 minutes later than thymol. These drugs were effective in half the time taken by sodium arsenite, sodium arsenite plus sulphate of copper or lysol. The worms were dead in thymol creosote and beta-naphthol when others were still swimming in sodium arsenite solutions. (6) Sodium arsenite is more effective than sodium arsenite plus sulphate of copper and more effective than lysol. Sodium arsenite plus sulphate of copper is as effective as lysol and picric acid. (7) Sulphate of copper comes a bad last, and worms died only just before those placed in the control medium of pure acacia solution.

(2) Resistance of Adult Worms in 0.05 Per Cent. Solution.

From the observation made previously under the heading "Passage of the Drugs," it can be deduced that in the majority of cases the bulk of the drug passes slowly from the rumen to the abomasum in a very dilute condition. In the observations recorded in the following table the solution of the drug was ten times weaker than that in the previous experiment:—

Drugs used in a 0.05 per cent. Solution.

Drug.	Index No.	TIME OF OBSERVATION.										
		30 Mins.	40 Mins.	50 Mins.	1 Hour.	1½ Hour.	2 Hours.	3 Hours.	4 Hours.	5 Hours.	6 Hours.	9 Hours.
Sodium arsenite.....	1	50 swim. 50 mov.	80 mov. 20 reac.	50 mov. 50 reac.	30 reac. 70 dead	—	Dead					
Creosote.....	1	Swim. —	50 swim. 50 mov.	20 swim. 80 mov.	20 swim. 80 mov.	—	60 reac. 40 dead	25 reac. 75 dead	10 reac. 90 dead	5 reac. 95 dead	Dead	
Thymol.....	1	Mov. —	50 mov. 50 reac.	50 mov. 50 reac.	20 mov. 80 reac.	—	Dead					
Sodium arsenite.....	2	50 swim. 50 mov.	—	—	50 reac. 50 dead	20 reac. 80 dead	Dead					
Creosote.....	2	Swim. —	—	—	50 mov. 40 reac. 10 dead	90 reac. 10 dead	70 reac. 30 dead	25 reac. 75 dead	15 reac. 85 dead	5 reac. 95 dead	Dead	
Lysol.....	2	80 swim. 20 mov.	—	—	50 swim. 50 mov.	50 mov. 40 reac. 10 dead	80 reac. 20 dead	Dead				
Thymol.....	2	50 mov. 50 reac.	—	—	90 reac. 10 dead	Dead						
Picric acid.....	2	50 swim. 50 mov.	—	—	50 mov. 50 reac.	50 reac. 50 dead	10 reac. 90 dead	(2½ Hours.) Dead				
Gasoline.....	2	Swim. —	—	—	Swim. —	Mov. —	80 mov. 20 reac.	10 reac. 90 dead	5 reac. 95 dead	Dead		

Drug.	Index No.	TIME OF OBSERVATION.										
		30 Mins.	40 Mins.	50 Mins.	1 Hour.	1½ Hour.	2 Hours.	3 Hours.	4 Hours.	5 Hours.	6 Hours.	9 Hours.
Sodium arsenite.....	3	50 swim. 50 mov.	— —	— —	30 reac. 70 dead	10 reac. 90 dead	Dead					
Sulphate of copper..	3	90 swim. 10 mov. —	— — —	— — —	50 swim. 50 mov. —	50 swim. 50 mov. —	20 swim. 60 mov. 20 reac.	10 swim. 80 reac. 10 dead	5 swim. 85 reac. 10 dead	50 reac. 50 dead	15 reac. 85 dead	5 reac. 95 dead
Sulphate of copper. } Sodium arsenite..... }	{ 3 }	70 swim. 30 mov.	— —	— —	30 swim. 70 mov.	90 reac. 10 dead	70 reac. 30 dead	10 reac. 90 dead	1 reac. 99 dead	Dead		
Lysol....	3	90 swim. 10 mov.	— —	— —	70 swim. 30 mov.	30 swim. 70 mov.	10 mov. 70 reac.	80 reac. 20 dead	80 reac. 20 dead	40 reac. 60 dead	20 reac. 80 dead	10 reac. 90 dead
Thymol.....	3	50 mov. 50 reac.	— —	— —	90 reac. 10 dead	50 reac. 50 dead	10 reac. 90 dead	(2½ Hours) Dead				
Beta-naphthol.....	3	50 mov. 50 dead	— —	— —	2 mov. 98 dead	Dead						
Picric acid.....	3	50 swim. 50 mov.	— —	— —	50 mov. 50 reac.	50 reac. 50 dead	30 reac. 70 dead	Dead				
Gasoline.....	3	Swim. —	— —	— —	Swim. —	Mov. —	80 mov. 20 reac.	10 reac. 90 dead	Dead			

Remarks.—(1) Experiment No. 1 (Index No.) was undertaken with worms obtained from a case of natural infection; the worms were all in the adult stage, but in different stages of development, the youngest just commencing oviposition. (2) Experiment No. 2 was made with worms obtained from a case of artificial infection produced sixty days previously. The worms were all fairly well developed, having started oviposition. (3) Experiment No. 3 was made with worms obtained from a case of artificial infection produced forty-five days previously. The worms were full grown and rather old, 95 per cent. being from the artificial infection, of which 50 per cent. were females in oviposition.

Conclusions.—(1) In the experiments reported in the above table the difference in ages amongst the batches of worms was not so sharply defined as to justify a distinction being made in the figures showing their resistance to the drug, but the general impression was gained that the degree of resistance stood in direct proportion to the age of the worm. (2) In the above table the poisonous effects of thymol (Experiment No. 1) and naphthol (Experiment No. 3) are nearly the same, although in judging from the figures after one hour's observation, naphthol appears the more destructive of the two. (3) Sodium arsenite shows a remarkable constancy in its effects. It is inferior to thymol in the first experiment, but superior in the third experiment after one hour's contact with the larvae. (4) Picric acid shows also a consistent but less destructive effect than sodium arsenite. (5) Sulphate of copper plus sodium arsenite and gasolene killed the worms after four to five hours' contact. (6) Creosote showed also a consistent action, but rather ineffective. (7) The remarkable effect of lysol in experiment No. 2 presumably depended upon some undetected factor, as it did not agree either with the result given in Experiment No. 3 (creosote) or with the effects reported in other tables. According to the results in Experiment No. 3, lysol and sulphate of copper were least effective.

General Conclusions on the action of Drugs on H.C. "in vitro."

(1) Mature larvae were more resistant than adult worms. (2) The resistance of adult worms appeared to increase with age. (3) In saturated solution sodium arsenite was the most effective on larvae. In a more diluted solution sodium arsenite was surpassed by creosote and lysol. (4) In a rather concentrated solution (0.5 per cent.) thymol, beta-naphthol, creosote, and lysol were the most effective on adult worms. Sodium arsenite was only about one-third or a quarter as effective. In weak solutions (0.05 per cent.) thymol and beta-naphthol were perhaps the most effective, but sodium arsenite stands very close, and shows a more consistent action. Creosote and lysol were not so effective. (5) In comparing the resistance of larvae to sodium arsenite and creosote, both at 0.5 per cent. and 0.05 per cent., it appeared that creosote in a dilution of 0.5 per cent. was much stronger than sodium arsenite, yet in a dilution of 0.05 per cent. was much weaker.

C.—MEDICINAL TREATMENT.

EXPLANATION OF ABBREVIATIONS USED.

G., gramme; mg., milligramme; H.C., *Haemonchus contortus*; *Oes. col.*, *Oesophagostomum columbianum*; *Bon. trig.*, *Bunostomum trigonocephalus*; *Tric. ins.*, *Trichostrongylus instabilis*; *Trich. ex.*, *Trichostrongylus extenuatus*; *Strong. pap.*, *Strongyloides papillosus* (Weld, 1856); m., male; f., female.

INFECTION THROUGH THE MOUTH AND INFECTION THROUGH THE RUMEN.

In the beginning of the experiments sheep were artificially infected with larvae of H.C. by collecting larvae from the walls of a jar which contained a culture of infected faeces. Cultures were always chosen in which a good colony was in active migration; the larvae were collected with a piece of moist blotting paper, the blotting paper then being immersed in a given amount of pure water. The suspension of larvae was then mixed with grass or bran and given to the sheep previously starved. It was, however, found that the distribution of larvae in a lot of sheep was not uniform, as some sheep eat more and some less. Consequently the suspension of larvae was directly injected into the rumen through the left flank by a Pravaz's syringe of 10 c.c. capacity. The advantages of the rumenal injection were found to be the following:—(1) Every sheep received the same degree of infection; (2) The infection can be performed in one day. (3) The infection can be graduated at will, viz.: knowing that 10 c.c. of suspension of larvae is the average heavy infection in a sheep, this can be increased or decreased according to the object.

In Experiment No. 53 (*re* "dietetic treatment") infection of 10 c.c. was also attempted via the mouth, with good results, but it was found that the liquid could go straight into the abomasum, and it was felt that in such cases larvae could be carried in a short time from the abomasum into the intestine without having time to undergo those changes which enable them to remain in the abomasum. In the veld the larvae ingested by a sheep pass into the rumen with the solid food.

Method of Treatment.—In the method of treatment referred to in the following experiments, sheep were frequently dosed for two or three consecutive days. This consisted in keeping the experimental sheep away from food and water for 12-17 hours before each dose, and for 2 to 5 hours afterwards. During treatment water was totally excluded when bluestone, Cooper's dip, sodium arsenite, or their combinations were administered. Unless otherwise stated, therefore, the administration of a dose of any vermicide implies in addition the standard dietetic treatment indicated.

Seasonal Conditions during Infection and Treatment.—In view of possible variations in results of both infection and treatment due to varying climatic conditions, it was considered advisable to report the date on which all experiments were undertaken. It may be remarked that in the Transvaal the summer months are usually considered to be from September to March, and it is during these months that the yearly rainfall occurs, averaging about 21 inches, and that the annual temperature is at its maximum. It is seldom that any appreciable fall of rain occurs during the remaining months of the year.

Sheep in Poor Condition.—As a general observation it may be remarked that in many cases sheep used in connection with the experiments detailed in this paper had previously been used for experiments in connection with blue-tongue vaccine. The production of this vaccine is one of the routine duties of this laboratory, necessitating the employment of approximately 2000 sheep per season, and to avoid expense in purchasing fresh sheep, use was often made of these animals discharged from vaccine preparation. Hence it must be remembered that such sheep were frequently in poor condition and anaemic when available for investigations, due to the heavy bleeding necessitated by the preparation of the vaccine. The following experiments give the details of the various drugs used as anthelmintics, the date on which treatment was commenced being given in each case.

TREATMENT WITH SINGLE DRUG.

(a) *Picric Acid.*—*Experiment No. 1* (7th March, 1912).—Ten sheep were dosed for three consecutive days with 0.250 grammes picric acid in 90 c.c. water. No disturbance was observed. The number of eggs counted in the droppings was 20-25 both before and after treatment.

Experiment No. 2 (13th March, 1912).—Four sheep were dosed with four different doses of picric acid, and the results are summarized in the following table:—

No. of Sheep.	Number of Eggs before Dosing.	Dose of Picric Acid.	Dissolved in Water.	NUMBER OF EGGS AFTER DOSING.				
				March.				
				14th	15th	16th	17th	18th
3873	50	Grammes.	c.c.					
		0.400	150	130	100	103	80	73
3914	50	0.500	150	32	64	60	59	60
3910	33	0.750	150	12	15	15	20	25
3903	36	1	250	30	30	70	50	43

NOTE.—Warm water was used to obtain a complete solution of the picric acid.

Experiment No. 2A (1st March, 1912).—Five sheep were dosed with 1 gramme picric acid, and the results were as follows:—

No. of Sheep.	NUMBER OF EGGS BEFORE DOSING.		Dosed with 1 gramme Picric Acid Dissolved in Water.	NO. OF EGGS AFTER DOSING.			
	March.			March.			April.
	18th	21st		22nd	23rd	25th	1st
			C.C.				
3878	25	30	250	60	14	13	10
3872	70	23	150	56	47	13	10
3871	140	100	200	200	276	30	45
3867	25	30	150	35	40	27	30
3866	96	98	200	120	126	80	40

Observations: (1) During the above experiment no ill effects were noted in the treated sheep. (2) Small doses repeated, or large doses up to 1 gramme of picric acid did not expel the worms. (3) It would appear from this experiment that under the effect of picric acid the worms lay a larger number of eggs.

(b) *Thymol*.—Seeing that good results were obtained with thymol in man against ankylostomiasis, the following experiments were undertaken:—

Experiment No. 3 (12th September, 1913).—Six lambs, about two months old, were dosed with 2 grammes of thymol, given in two doses with an interval of an hour, and repeated for three days. The lambs were kept without food for twelve hours before dosing and two hours after dosing. No ill effects were observed from the treatment. Before dosing, cultures from the droppings were made and some larvae were detected in each of the six lambs. After dosing, cultures from the droppings were made on the third and tenth days, and the cultures from one lamb only showed larvae.

Experiment No. 4 (12th September, 1913).—Three sheep (6-tooth) were dosed with 3.5 grammes of thymol for three consecutive days. No ill effects were noted from this treatment. In cultures from droppings, larvae were noted both before treatment and four days later. On the 24th of the same month these three sheep were dosed with 5 grammes of thymol, and the dose was repeated for three consecutive days. On the third day after the last dose a purgative of 25 grammes of magnesium sulphate was also given. In the cultures made from droppings on the third and seventh days after treatment some larvae were present.

Experiment No. 5 (20th April, 1915).—Five sheep (8-tooth) were dosed with 3 grammes of thymol. The dose was repeated on the 21st and 22nd. After the last dose a tablespoonful of sodium sulphate was also given. Larvae in cultures of droppings were found before and after treatment. (21th April, 1915).—Ten sheep were dosed in a similar way, but with a dose of 4 grammes of thymol. To obtain a definite conclusion on the result of the treatment, some of the above sheep were killed and the stomachs and intestines were thoroughly

examined for nematodes, the results being given in the following table:—

No. of Sheep.	Number of Eggs before Dosing.	Daily Dose of Thymol.	Number of Eggs after Dosing.	H.C. found in the 4th Stomach.	<i>Oes. col.</i> found in Large Intestine.
		Grammes.			
7237	3	3	8	6 m.	8
7254	6	3	—	—	—
7245	3	3	—	—	—
7218	20	3	3	21 m. & f.	6
7273	6	3	13	5 f. & 25 m.	7
7223	120	4	4	Very numerous	3
7258	160	4	4	" "	Numerous
7206	5	4	—	—	—
7283	6	4	6	10 f., 4 m.	Nil
7176	3	4	—	—	—

NOTE.—For further observations on the vermicial effect of a "double dose" of thymol, where the results were unsuccessful in all three lambs treated, see Experiment No. 49, paragraph "Comparative Experiments on the Therapeutic Value of some more Common Drugs against Wire-worm Infection."

Conclusions.—(1) No ill effects were noted in dosing lambs with 2 grammes of thymol or adult sheep with 5 grammes of thymol for three consecutive days. (2) The above treatment with thymol does not appear to be successful against either H.C. or *Oes. col.* infection.

(c) *Beta-Naphthol.*—*Experiment No. 6.*—The five sheep that were used on the 20th April, 1915, in the experiment with thymol (see Experiment No. 5) were dosed with 6 grammes of beta-naphthol for three consecutive days, plus 30 grammes of magnesium sulphate on the third day. As was mentioned in Experiment No. 5, larvae were still present in the droppings of the sheep after the second treatment with thymol. Cultures from the droppings made four and fourteen days after treatment with beta-naphthol gave negative results. In the year 1914 beta-naphthol was occasionally used at the Laboratory for treating lambs infected with H.C. The dose used was 2 to 4 grammes, repeated for three days. Cultures made from droppings after treatment usually gave negative results. In 1915 more exhaustive experiments were undertaken to ascertain the value of the beta-naphthol treatment against wire-worm infection, and for this purpose it was thought advisable to ascertain the maximal dose for single and repeated administrations.

Experiment No. 6A.—As it appeared from Experiment No. 6 that the dose of 6 grammes was well supported for three consecutive days, the following experiment was carried out:—Three sheep were dosed with 8 grammes and three other sheep with 10 grammes of beta-naphthol. Two hours later two of the sheep that were dosed with 8 grammes showed a small amount of foam at the mouth, and one of the sheep dosed with 10 grammes was lying down showing foam at the mouth, but recovered in the afternoon. The rest of the sheep showed no ill effects.

Experiment No. 6B (9th March, 1915).—For the purpose of seeing whether the disturbance showed by the three sheep in Experiment No. 6 is a toxic one, ten sheep in rather poor condition that some

days previously had passed through a heavy bleeding for the production of blue-tongue vaccine were selected. These ten sheep were kept for seventeen hours without food, and on the 10th March, 1915, at 9.30 a.m., were each dosed with 10 grammes of beta-naphthol. Eight of them died of poisoning either on the day of treatment or on the following day, and the remainder were killed for the sake of experiment. The result of the drug on the H.C. and other helminths of the digestive canal is given in the following table:—

Time of Death.	Worms in Abomasum.	Worms in Intestines.
10.3.16 11 a.m. 12.30 p.m. 2 p.m. 2.30 p.m. 4 p.m. 6 p.m. 10 p.m.	30 H.C. living 20 H.C. " 20 H.C. Some with slow movement ; some motionless Numerous H.C. living ; some showed very slow movements Some H.C. living and showing slow movements Some H.C. living Some H.C. dead (see remark 1)	Large intestine : numerous <i>Oes. col</i> living.
11.3.15 2 p.m.	150 H.C., viz.: 90 dead, 59 with slow movements, 1 quite lively	Jejunum : numerous dead H.C.
17.3.15 9 a.m. (killed)	14 H.C., viz.: 10 f. and 4 m. living (see remark 2)	Jejunum: <i>Bon. trig.</i> Large intestine : <i>Oes. col.</i>
17.3.15 9 a.m. (killed)	30 H.C. in good condition	Duodenum : frequent <i>Tric. ins.</i> Jejunum : three <i>chabertia</i> . Large intestine : 10 <i>Oes. col.</i>

Remarks.—(1) The post-mortem on the sheep which died at 10 p.m. on the 10th March, 1916, was held the next morning. (2) The droppings contained in the rectum of the sheep which was killed on the 17th March, 1915, were put in cultures, and after four days rare larvae of *strongyli* and numerous larvae of *Strong. pap.* were found.

Experiment No. 6c (22nd March, 1915).—Judging from Experiments 6 and 6A, the maximal dose for three consecutive days would appear to be 6 grammes. Accordingly ten adult sheep that fifteen days earlier had been bled for blue-tongue vaccine were kept without food and water for seventeen hours, and then dosed with 6 grammes of beta-naphthol. One hour later three showed dyspnoea with foam at the mouth. Three hours after treatment one sheep died. No worms were found in the abomasum on post-mortem. During the same night a second sheep died. The surviving sheep were not dosed again.

Experiment No. 6D (19th April, 1915).—Ten adult sheep in poor condition, taken off the veld, were starved for seventeen hours and then dosed with 4 grammes of beta-naphthol. The sheep were submitted to the usual dietetic treatment, and on the 20th April, 1915, the second dose of 4 grammes of beta-naphthol was given. Two hours later one sheep showed symptoms of poisoning, dying the following

day, when a second sheep also died. In the first sheep on post-mortem the abomasum showed numerous adult H.C., 50 per cent. of which were living. Numerous larvae, the majority in good condition, were sheltered in the blood coagula. Living *Oes. col.* were found in the large intestine. In the second sheep the post-mortem examination was delayed, and in the abomasum fifteen dead H.C. were found. In the large intestine were numerous dead *Oes. col.* The third dose was not given to the surviving sheep. On the 22nd April, 1915, a third sheep died. On post-mortem the abomasum did not contain worms. In the large intestine were numerous living *Oes. col.*

NOTE.—For the vermicial effect of beta-naphthol on wireworm infection see also Experiment No. 49 (paragraph "Comparative Experiments on the Therapeutic Value of some more Common Drugs against Wire-worms"), where it was ineffective on all three sheep treated with a double dose.

Conclusions.—(1) The single dose of 8-10 grammes of beta-naphthol was highly fatal to sheep, and was not completely effective against wire-worm infection.

(2) Sheep showed a highly variable sensibility to repeated doses, and not all the worms were killed with doses which proved to be fatal for the host.

It is the impression of the writer that from the above experiments the variable sensibility of sheep to beta-naphthol cannot be adequately explained by the condition of health of the sheep, but probably is connected with their individual susceptibility. From a practical point of view beta-naphthol could not be used with safety by farmers, and the vermicial effect on wire-worm infection would not be successful.

(d) *Sulphate of Copper (Bluestone).*—Many experiments with the above remedy in one dose only have already been undertaken and the results published by Sir Arnold Theiler.

Experiment No. 7.—Five sheep were dosed for three days consecutively with 650 mg. (10 grains) of bluestone. On the third day the treatment was followed by the administration of 10 grammes of sodium sulphate. Two sheep were killed a few days after treatment, and the result is given in the following table:—

No. of Sheep.	Result of Examination of Cultures before Dosing.	Nematodes found in the Digestive Tract on Post-mortem.
7071	Larvae numerous ...	—
7062	" " " ...	45 adult H.C. and numerous <i>Oes. col.</i>
7029	Larvae rare ...	—
6494	" " " ...	20 adult H.C. and numerous <i>Oes. col.</i>
6755	Larvae numerous ...	—

Conclusions.—The dose of 650 mg. of bluestone in powder form given over three successive days was unsuccessful against wire-worms in both sheep. In view of this result it was not thought advisable to undertake any other experiments with bluestone alone.

General Conclusions on the Dosing Experiments with a Single Drug.—From the results of dosings with picric acid, beta-naphthol, thymol, and bluestone, the opinion was formed that a single vermicial

substance, even when administered in a fatal dose, is never completely successful against wire-worms. This opinion has been already supported by many writers in veterinary science.

TREATMENT WITH A COMBINATION OF DRUGS.

The following experiments are devoted to the combined Chemotherapy or Polypharmacy against wire-worm infection:—

Arsenical Compounds and Bluestone.—As already mentioned, Theiler's earlier experiments were carried out with arsenic in the form of arsenious oxide, and also with arsenic in soluble form, as it occurs in Cooper's dip.* Cooper's dip was then selected as being universally procurable in convenient packet form by farmers throughout the Union. In many of the earlier trials, this product (a proprietary complex containing arsenite, sulph-arsenite, and excess of free sulphur) was also used, but in later experiments recourse was taken to sodium arsenite, on the ground that, as a single chemical compound sold on a basis of arsenious oxide content, and free from ambiguity in nomenclature, it is more suitable as a raw material in making up a composite wire-worm remedy. Theoretical considerations suggest that valency and degree of solubility are the two main factors governing the toxicity of inorganic arsenic compounds, and chemically equivalent quantities of arsenic as arsenite of soda and as Cooper's dip should, therefore, exercise much the same effect upon both wire-worms and sheep. Since the former contains approximately four times the amount of soluble arsenious oxide as the latter, 125 mg. of arsenite of soda was taken as equivalent to 500 mg. of Cooper's dip, and comparison between experiments involving dosing of sheep with both commodities was, therefore, made on this basis.

(a) *Cooper's Dip and Sulphate of Copper (Bluestone).* (Dose repeated for three consecutive days.)

SUMMARY (*Experiments Nos. 8-13*).—Sir Arnold Theiler recommended for sheep of four to eight tooth a single dose of 10 grains of Cooper's dip and 10 grains of bluestone. On the basis of this recommendation, the object of Experiment No. 8 was to find out whether the dose of 650 mg. of Cooper's dip and 650 mg. of bluestone would be tolerated by adult sheep when given for three consecutive days, and it proved to be safe in all five sheep. In Experiment No. 9 the same treatment was repeated with success on four anaemic sheep. Experiment No. 10 is a repetition of Experiment No. 9, but the droppings of the sheep were examined before treatment for eggs of H.C. The sheep withstood the dose, and its complete effect against H.C. was proved on post-mortem, with the exception of one sheep, in which some males of H.C. were found. Experiment No. 11 was made with the object of controlling Experiment No. 10, but in this the six sheep and a lamb used were artificially and heavily infected with wire-worms. The post-mortem examination on four sheep killed after treatment demonstrated the complete effect of the treatment. For

* Agriculture Dept. Bulletin No. 17 of 1912, Union of South Africa.

Agriculture Dept. Bulletin No. 48 of 1912, Union of South Africa.

Agriculture Dept. Bulletin No. 63 of 1912, Union of South Africa.

"Experiments to determine the safe dose of White Arsenic, Cooper's Dip, and Bluestone for Sheep."

"Interim report on the dosing of Sheep with Cooper's Dip and Bluestone under the conditions of a Sourveld farm."

"Wire-worms in Sheep and their Treatment."

the purpose of testing the treatment on a large scale, in Experiment No. 12 fifty sheep in poor condition taken off a farm were dosed as in Experiment No. 8. The post-mortem of four of the poorest sheep and of one in good condition showed the complete effect of the treatment. Seventeen other sheep which were available at the time were also treated with the same method, and with the exception of some anorexia, no ill effects were noted. Experiment No. 13 was carried out for the same purpose as Experiment No. 12, but this time the mortality was unexpectedly heavy. It was then thought advisable to reduce the treatment to one dose given on two consecutive days.

Details of Experiments.—*Experiment No. 8* (22nd March, 1915).—On ten anaemic sheep, which fifteen days previously had passed through a blue-tongue reaction and had been submitted at the time to a heavy bleeding; 650 mg. of bluestone, plus 650 mg. of Cooper's dip in dry powder form, was given for three consecutive days to five of the above sheep, according to the method already described. The other five sheep received only the first dose, and were kept as controls. None of the ten sheep showed any ill effects either during or after treatment. *Experiment No. 9*: In March, 1915, when the infection of H.C. amongst the sheep at the Laboratory was very heavy, four anaemic sheep (6-tooth) were selected, and the droppings were examined for eggs; on the 27th March, 1915, three of the sheep were treated with 650 mg. of Cooper's dip powder, plus 650 mg. bluestone in powder form, for three consecutive days. On the last day of treatment 8 to 10 grammes of mag. sulph. were also given with the vermifuge. The fourth sheep (No. 6980) received the full dose on the first day of treatment, and on the two following days received 650 mg. of bluestone only. The results are summarized in the following table:—

No. of Sheep.	Examination of Cultures of Larvae from Droppings.	Date of Post-mortem Examination.	Species of Worms found.
6088	Fairly numerous	13/4/15	No H.C. Few <i>Tric. ins.</i> in the small intestine. Numerous <i>Oes. col.</i> in caecum and colon.
7040	"	13/4/15	No H.C. Few <i>Tric. ins.</i> Few <i>Oes. col.</i> in caecum and small intestine.
6500	Numerous	15/4/15	No H.C. Three <i>Bon. trig.</i> in small intestine. Few <i>Oes. col.</i> in caecum.
6980	"	15/4/15	Numerous H.C. in the 4th stomach. Four <i>Bon. trig.</i> in small intestine.

NOTE.—The above treatment apparently killed all the H.C. in the sheep, as No. 6980, which served as a control, showed numerous wire-worms. The treatment did not have any effect on the other nematodes present in the intestines.

Experiment No. 10.—Ten anaemic sheep (6 to 8 tooth), apparently suffering from haemonchosis, were examined for eggs of H.C., and treated with the same dose on the 19th-21st April, and in the same manner as in Experiment No. 9. The sheep which showed a large number of eggs before treatment were killed at different intervals, and

the alimentary tract carefully examined for nematodes. The results are given in the following table:—

No. of Sheep.	Number of Eggs in Faeces.	Date of Post-mortem.	Species of Worms found.
7295	83	24/4/15	No H.C. Two <i>Bon. trig.</i> in small intestine. 48 <i>Oes. col.</i> in caecum and colon.
7241	150	27/4/15	No H.C. One <i>Bon trig.</i> in small intestine. 21 <i>Oes. col.</i> in caecum and colon.
7264	18	28/4/15	50 H.C. males in the stomach. No worms in the intestine.
7148	90	28/4/15	No H.C. No worms in the intestine.
7262	70	1/5/15	No H.C. 15 <i>Oes. col.</i> in colon.
7306	6	1/5/15	No H.C. 20 <i>Oes. col.</i> in colon.
7268	4	—	—
7280	90	3/5/15	No H.C.
7313	4	—	—

NOTES.—It seems that in the case of Sheep No. 7624, although eggs were present in the faeces before treatment, no female H.C. were found on post-mortem, yet males of the same species were seen, thus indicating that the female H.C. were killed by treatment. It cannot be accepted that the eggs found previous to dosing were those of *Oes. col.*, since the results of Experiments Nos. 9 and 10 show conclusively that *Oes. col.* is not killed by Cooper's dip and bluestone. The fact that the males of H.C. show a greater resistance than the females was also occasionally observed with specimens that were collected whilst in good condition and kept alive in water, when the males survived longer than the females. Sheep No. 7280 was very anaemic when treatment was started, appearing from the clinical appearance to be heavily infected. Treatment was tolerated, but the sheep died on the 3rd May, 1915. On post-mortem examination the appearance was typical of anaemia due to verminosis, but no wire-worms were present. From the above experiment it appears that the treatment was decidedly effective against H.C.

Experiment No. 11.—In order to see if the treatment used in the above three experiments was successful in a heavy infection, the following experiment was carried out:—On the 4th June, 1915, six sheep (6-tooth), and in fairly good condition, were infected with a large number of H.C. On the 12th June, 1915, one of the infected sheep, No. 7208, was treated according to the method used in the above three experiments, with the object of finding out if the treatment was also effective against larvae of H.C. that presumably were still sheltered in the coagula. On the 1st July, 1915, one of the infected sheep was killed (No. 7247), and the fourth stomach was found to be swarming with H.C. in the adult stage, but not yet in the period of oviposition, whereas the other sheep showed a few eggs in the droppings. The examination of the droppings was repeated from time to time until the 12th July, 1915, when about 50 eggs per smear were detected. One of the four remaining sheep died accidentally whilst being dipped. Treatment with the same dose and by the same method as mentioned in the above three experiments was carried out on the 12th, 13th, and 14th July on the three remaining sheep, Nos. 7198, 7208, and 6048. During the same three days, lamb No. 7325, 6 months old, which had been heavily

infected with larvae twelve days previously, was also treated according to the above method, but the dose used was Cooper's dip 300 mg. and bluestone 300 mg. The results were as follows:—

No. of Sheep.	Date of Post-mortem Examination.	Species of Worms present.
6048	15.7.15	No worms present in the alimentary canal.
7208	17.7.15	" " " " " "
7198	21.7.15	No worms in the fourth stomach. Few <i>Oes. col.</i> in caecum and colon.
Lamb 7325	16.7.15	No worms in the fourth stomach. 12 <i>Oes. col.</i> in colon and caecum.

NOTES.—During the time of infection the sheep and lambs were well fed to avoid death from anaemia. On post-mortem examination a fair amount of fat was present, but the anaemia and hyperaemia were typical of verminosis. Sheep No. 7201 was not killed, as it was in good condition and no larvae were found in cultures.

Experiment No. 12 (23rd July, 1915).—With the object of testing the above treatment on a more extensive scale, fifty sheep from the farm were submitted to treatment. Nineteen of the sheep were 8-tooth, thirty-one were 6-tooth. Some of the sheep were in poor condition. In a lot of nineteen, mag. sulph. was not administered on the third day of treatment. None of the treated sheep showed any ill effects during or after treatment. Four sheep in very poor condition and one in good condition were killed to ascertain the effect of the treatment. The results were as follows:—

No. of Sheep.	Date of Post-mortem Examination.	Species of Worms present.
7296	28.7.15	No worms present.
7329	29.7.15	No H.C. present. Two <i>Bon. trig.</i> in small intestine.
7288	29.7.15	No H.C. present. Few <i>Oes. col.</i> in colon.
7275	29.7.15	No worms present.
7311	31.7.15	No H.C. present. Few <i>Oes. col.</i> in colon.

NOTES.—Sheep Nos. 7296 and 7329 were selected from the lot to which no purgative was given on the third day of treatment.

Experiment No. 12A (5th August, 1915).—Seventeen sheep, of which ten were 4-tooth and seven were 6-tooth, were dosed with the above method. 2 p.m.: one 4-tooth sheep was found separated from the flock and refused food. (6th August, 1915)—2 p.m.: none of the sheep were feeding well. No other ill effects were observed.

Experiment No. 13 (19th August, 1915).—A mixed lot of Merino and Persian sheep, numbering seventy-two—among which were three 2-tooth, sixteen 4-tooth, and thirty-eight 6-tooth, some being in good condition (the others in poor condition), and some were pregnant ewes—were treated for three consecutive days with 650 mg. of Cooper's dip and 650 mg. of bluestone in powder. The result was as follows:—(20th August, 1915.) A Merino ewe, 6-tooth, in very poor condition, died before the second dose was given. The pathological anatomical diagnosis showed a marked marasmus and some degree of congestion in the lung and spleen which was connected with arsenical poisoning.

(21st August, 1915.) A 4-tooth Merino ewe in fairly good condition died before the third dose was given. The pathological anatomical diagnosis was acute arsenical poisoning. (22nd August, 1915.) An 8-tooth Merino ewe in poor condition died in the morning, and on post-mortem examination showed lesions of arsenical poisoning. (24th August, 1915.) An 8-tooth Merino ewe in good condition died, showing on post-mortem examination gastro-enteritis due to arsenical poisoning. (24th August, 1915.) A 6-tooth Persian ewe in good condition died from gastro-enteritis due to arsenical poisoning. (25th August, 1915.) A 6-tooth Merino ewe in poor condition died, showing marked anaemia and marasmus. (25th August, 1915.) A 6-tooth Persian ewe in good condition died with extensive marked hyperaemia of the colon and caecum, probably due to arsenical poisoning. (29th August, 1915.) An 8-tooth Merino ewe in poor condition died of pleuro-pneumonia. (29th August, 1915.) An 8-tooth ewe in fair condition died of pleuro-pneumonia.

Conclusions.—(1) The result of the above experiment suggests that there were some peculiarly unfavourable conditions either in the sheep or in the drugs that were not detected at the time of dosing, seeing that ten deaths occurred out of seventy-two sheep, whereas in the five previous experiments the mortality was nil. (2) The fact that the ten dead sheep were all ewes suggests the conclusion that ewes are more susceptible to arsenic than males.

General Conclusions on Experiments Nos. 8 to 13.—(1) A mixture of Cooper's dip and sulphate of copper in the dose of 650 mg. of each, given for three consecutive days, proved effective against H.C. in sheep. (2) In the one negative effect in Experiment No. 10 such a sheep would not be a "reservoir" of infection on a farm as the H.C. were all males and must gradually die out. (3) The treatment did not prove effective against other nematodes of the small and large intestine. (4) Although the dose tested in the experiments above does not produce any appreciable ill effects in the majority of cases, it would not be advisable under the usual farming conditions as the mortality might be heavy.

(b) *Cooper's Dip 650 mg. and Sulphate of Copper 650 mg.* (Dose repeated for two consecutive days.)

Summary of Experiments Nos. 14 to 26.—Experiment No. 14 was undertaken for the purpose of testing on a small batch of sheep the combined effect of the two days' treatment on the host and on the worms at the same time. Experiment No. 16 had a similar object as Experiment No. 14, but on lambs, and at a period when the H.C. are sheltered in the blood coagula of the abomasum. Seeing the satisfactory results obtained from the two previous experiments, it was thought advisable to test the resistance of sheep to the dose of 650 mg. on a more extensive scale. In the Experiments Nos. 17 to 25 sheep usually in poor condition and suffering from anaemia were treated for two days with a dose of 650 mg. of Cooper's dip and bluestone, except in Experiments Nos. 19 and 25, in which the dose of 500 mg. of bluestone and 650 mg. Cooper's dip was tested. As the dose was well supported it was concluded that the mixture of 650 mg. of Cooper's dip, plus 650 mg. bluestone, given for two days, was completely effective on the wire-worm infection and safe for the sheep. The next experiment was to see if the dose of 650 mg. of each could be reduced

without losing its efficacy. In Experiment No. 26 the dose of 650 mg. of each compound and of 500 mg. were compared in a batch of heavy infected sheep, and the dose of 500 mg. proved as effective as that of 650 mg. It was then decided to work in further experiments upon the dose of 500 mg. of bluestone and Cooper's dip respectively.

Details of Experiment No. 14 (20th October, 1915).—Seven sheep from 4 to 8 tooth and one lamb seven months old were infected by mixing mature larvae with the food. (29th November, 1915.) Cultures from the droppings of the infected sheep were all positive and rich in larvae. On the 30th November, 1915, and 1st December, 1915, the sheep were dosed with 650 mg. of Cooper's dip and 650 mg. bluestone powder. The lamb was dosed with 300 mg. of Cooper's dip and 300 mg. of bluestone in powder. During treatment the sheep and lamb did not show any ill effects. Five sheep and the lamb were killed after treatment, the results being as follows:—

Date.	Age of Sheep.	Condition.	Abomasum.	Caecum and Colon.	Cultures made from Faeces collected at Post-mortem Examination.
6.12.15	4-tooth	Fat—anaemic	No H.C.—Coag.	5 females of <i>Oes. col.</i>	Very rich in larvae; <i>St ong. pap.</i>
7.12.15	8-tooth	Good "	" "	No worms	Few larvae of Strongy- lides.
8.12.15	6-tooth	Good "	" "	No worms— parasitic nodules.	" "
9.12.15	4-tooth	Poor "	" "	8 females of <i>Oes. col.</i>	Good culture—one-third <i>Oes. col.</i> , two-thirds <i>Strong. pap.</i>
9.12.15	4-tooth	Good "	" "	No worms	—
8.12.15	Lamb	—	H.C. in 4th stage in numerous coagula. 4 old males of H.C.	" "	" "

NOTE.—“Coag.” in the column headed Abomasum indicates that remnants of old blood coagula were found adhering to the mucosa of Abomasum.

Conclusions from Experiment No. 14.—(1) The method of treatment proved completely effective for the adult sheep. (2) The fact that remnants of old coagula were found in four sheep out of five killed shows that the infection was a heavy one, and this is also proved by the cultures made before treatment. (3) In the lamb infected artificially, treatment was effective except on four adult males that presumably had sheltered in some coagula.

Experiment No. 16A (13th December, 1915).—Four lambs eight months old were heavily infected with larvae of H.C. suspended in water. (21st December, 1915.) Two lambs died with haemorrhagic gastritis and enteritis. No H.C. were detected in the abomasum. The contents were liquid and putrid. (29th December, 1915.) The two other lambs were sick and in very anaemic condition, and both were treated for two consecutive days with 300 mg. of Cooper's dip and 300 mg. of sulphate of copper. The treatment was rather severe for the two lambs, but both survived. (6th to 8th January, 1916.)

Lambs Nos. 8464, 8465, 8466, 8468, 8472, and 8473, aged from 3 to 7 months, in good condition, were fed with bran mixed with mature larvae of H.C. (12th January, 1916.) Lambs were dull, rather poor, and had diarrhoea. The remainder of the experiment is summarized in the following table:—

Date.	No.	Age.	Condition before Death.	Cause of Death.	Abomasum.
12.1.16	8473	5 Months	Dull—diarrhoea	Pneumonia ...	50 adult old H.C., numerous H.C. in the 4th stage—coag.
22.1.16	8470	6 Months	Sick—diarrhoea	Septic peritonitis	Few adult old H.C., fair number of H.C. in the 4th stage—coag.
30.1.16	8472	5 Months	Poor—anaemic ...	Haemonchosis ...	Few adult old H.C., numerous in the 4th stage—coag.
1.2.16	—	Treatment	—	—	—
2.2.16	—	"	—	—	—
12.2.16	8464	6 Months	Poor—anaemic ...	Killed ...	A few H.C. in the 3rd stage—few coag, a few <i>Tric. ins.</i> in adult stage.
12.2.16	8468	6 Months	" " ...	" ...	A few H.C. in the 3rd stage—few coag, a few <i>Tric. ins.</i> in adult stage.
12.2.16	8465	8 Months	Fair—anaemic ...	" ...	8 H.C. females which have just reached the 5th stage. Remnant of old blood coagula on the mucosa.
12.2.16	8466	8 Months	Fair ...	Not killed ...	—

Remarks.—The experiment had the object of finding out whether it is possible to completely remove the H.C. infection from young lambs at the time when the H.C. are sheltered in the coagula on the mucosa of the abomasum, consequently the treatment was undertaken before the H.C. had reached the stage of maturity. The abbreviation "coag." in the above table indicates that coagula were very numerous, and in one case (8472) coating more than half of the abomasal walls. (2) The dose given in the two days of treatment was Cooper's dip 300 mg. and sulphate of copper 300 mg. in powder. (3) The three lambs which died before treatment serve as controls to show the heavy infection resulting from the artificial administration of larvae. (4) In Nos. 8464 and 8468 the larvae of H.C. found at the post-mortem examination in the third stage were from an infection subsequent to the treatment. This can be seen when comparing these two lambs with the case of the three lambs out of the same batch that died a month earlier, where the larvae were already in the fourth stage. The *Tric. ins.* might also be from an infection after treatment, as it was found in other experiments, not yet recorded, that the parasitic larval period was very short, but this period has not yet been fully determined. It is equally likely that the *Tric. ins.* infection occurred

before treatment, as cases will be recorded later in this paper where these parasites resisted treatment. The few H.C. found in sheep No. 8465 were probably picked up previous to treatment and had apparently been deeply sheltered in some coagula.

Conclusions from Experiment No. 16.—(1) Lambs heavily infected with young H.C. may die without showing worms in the abomasum. This occurs when, in consequence of a marked gastritis, abnormal fermentations of the abomasal contents kill the parasites. The possibility that a gastro-enteritis was present in the batch of lambs can be excluded, as four other lambs in contact did not show any disease. (2) The treatment for two consecutive days of 300 mg. of Cooper's dip, plus 300 mg. of sulphate of copper, was not fatal for nine lambs of 4 to 8 months old, but in acute gastritis resulting from wire-worm infection this treatment can produce ill effects. (3) The treatment was effective on larvae of H.C. sheltered in coagula in two cases out of three.

Experiments Nos. 17 to 25.—A series of experiments was undertaken, when material was available at the station, to test more extensively, and on anaemic sheep in poor condition, the dose of 650 mg. of Cooper's dip, plus 650 mg. of bluestone. The results can be summarized in the following table.

Experiment.	Date.	No. of Sheep.	Age of Sheep.	Died or Killed.
17	29/30.9.15	4 6	4-tooth 6-tooth	
18	9/10.11.15	1 6 14 2	2-tooth 4-tooth 6-tooth 8-tooth	18.11.15. One 4-tooth very weak; killed; no H.C.
20	22/23.11.15	20 5	4-tooth 6-tooth	
21	17/18.12.15	2 14 24	8-tooth 6-tooth 4-tooth	10.12.15. Sheep were bled heavily for blue-tongue vaccine. At the time of treatment sheep were very anaemic and weak, whilst some were very thin. One 6-tooth groaning on the 18.12.15. After treatment was ill; on the 28.12.15 was killed. No H.C. and no inflammation in the abomasum.
22	22/23.12.15	25 15 10	4-tooth 6-tooth 8-tooth	Sheep were bled heavily for blue-tongue vaccine 7 days previously and were in a fair condition at the time of treatment.
23	25/26.12.15	30 17	4-tooth 6-tooth	Sheep were bled heavily six days previously and at time of treatment were in very poor condition. One 4-tooth (one of the three sheep in poor condition) was killed. No H.C. in abomasum and no lesions of poisoning. 4.2.16. One 4-tooth: no H.C., but gastro-enteritis noted. One 6-tooth: no H.C., but marasmus.
24	4/5.1.16	1 27 21 1	2-tooth 4-tooth 6-tooth 8-tooth	

Conclusions from Experiments Nos. 17 to 25.—(1) The dose of 500 mg. of Cooper's dip, plus 500 mg. of bluestone, given on two consecutive days did not cause any ill effects on forty-nine sheep. (2) The dose of 500 mg. Cooper's dip, plus 500 mg. of bluestone, for three consecutive days did not cause any apparent ill effects on ten sheep. (3) The dose of 650 mg. of Cooper's dip, plus 650 mg. bluestone, for three consecutive days did not cause any deaths and was well tolerated by 239 sheep. All the sheep used were anaemic and in poor condition.

Experiment No. 26.—Sheep Nos. 8885 (4-tooth), 8468 (6-tooth), 8368 (6-tooth), 8693 (4-tooth), 8227 (4-tooth), 8510 (4-tooth), all in fairly good condition, were infected on the 17th, 18th, 19th, and 20th of January, 1916, with larvae of H.C. mixed with bran. (1st February, 1916.) Sheep were all more or less diarrhoeic. From this date the sheep went through a progressive anaemia and loss of condition until the time of treatment. (6th February, 1916.) No. 8368 died of typical pronounced haemonchosis. The treatment was undertaken ten days before the arranged date as the majority of sheep appeared likely to die shortly. (10th February, 1916.) Nos. 8693, 8885, and 8227 were dosed with 650 mg. of Cooper's dip, plus 650 mg. of bluestone for two consecutive days, and Nos. 8418, 8510, and 7757 dosed with 500 mg. of Cooper's dip, plus 500 mg. of bluestone for two consecutive days.

The sheep were all killed after treatment. The result of the experiment is summarized in the following table:—

Date.	No. of Sheep.	Treatment (on 10/11.2.16).	Result.
6.2.16	8368	Died before treatment	Marked acute anaemia. Abomasum: parasitic haem extravasations in the sub-mucosa. Young H.C. very numerous.
11.2.16	7757	Died after first dose	Acute anaemia and marasmus. Abomasum: numerous young H.C. 75 per cent. dead, 25 per cent. swimming.
17.2.16	8693	650 C.D. + 650 B.S.	Killed: acute anaemia. Abomasum: no H.C. present.
17.2.16	8227	" "	Killed: acute anaemia. Abomasum: no H.C. present.
18.2.16	8885	" "	Killed: acute anaemia. Abomasum: no H.C. present. Colon: <i>Oes. col.</i>
17.2.16	8510	500 C.D. + 500 B.S.	Killed: acute anaemia. Abomasum: no H.C. present.
18.2.16	8408	" "	Killed: acute anaemia. Abomasum: no H.C. present. Colon: <i>Oes. col.</i>

NOTES.—Cultures were not made from the faeces as the sheep were suffering from diarrhoea. The fact that the sheep were steadily falling off in condition, were anaemic, had marked diarrhoea, and two died is conclusive evidence of H.C. infection. (2) The death of No. 7757 following the first dose was due to extreme anaemia, and the presence of living H.C. proved that one dose only is not sufficient to kill all the worms.

Conclusions from Experiment No. 26.—The administration of Cooper's dip, plus bluestone, for two days in the dose of 500 mg. each, and also in the dose of 650 each, was completely effective on sheep in the last stage of very strong haemonchosis.

(c) *Sodium Arsenite plus Sulphate of Copper.*

(1) *Average dose of sodium arsenite plus sulphate of copper for two consecutive days—Summary of Experiments Nos. 28, 29, 31, and 41.*—In Experiment No. 28 sodium arsenite plus bluestone was compared with Cooper's dip plus bluestone in heavily infected sheep. As the result was completely successful in both treatments, a series of control observations was made in Experiments Nos. 29 and 31, and the result was again successful. Experiment No. 41 had the object of ascertaining whether the H.C. could also be killed when sheltered in the coagula of the abomasal mucosa. The result was favourable from the farmers' point of view. Another observation made in Experiment No. 35 (see minimum effective dose) is quoted, in which a sheep treated with the minimal effective dose was completely freed from worms sheltered in the coagula. An experiment was also made to see whether sodium arsenite alone was completely effective for wire-worms. The details of this observation will be given later in Experiment No. 32, when dealing with the treatment of lambs with sodium arsenite. In this experiment some of the infected sheep were treated with sodium arsenite, plus bluestone, and others with sodium arsenite alone. The experiment proved that sodium arsenite alone is not completely effective for wire-worms in the doses confined to the limits of safety.

Details of Experiment No. 28.—On the 14th, 15th, and 16th February, 1916, twelve sheep in fair condition were infected with larvae mixed with bran. Three of them died from haemonchosis, five were treated with 125 mg. sodium arsenite plus 500 mg. bluestone, whilst the remaining four received 500 mg. Cooper's dip plus 500 mg. of bluestone. The results are summarized in the following table:—

No. of Sheep.	Treatment.	Date of Treatment.	Date of Death.	Result of Post-mortem Examination.
8331	Not treated	—	18.3.16	Acute haemonchosis. H.C. very numerous.
8395	"	—	18.3.16	" " "
9402	"	—	18.3.16	" " "
8616	Sodium ars., 125 mg. Bluestone, 500 mg.	21/22.3.16	1.4.16 (killed)	Not a single H.C. " Marked anaemia.
8325	Sodium ars., 125 mg. Bluestone, 500 mg.	"	11.4.16 (killed)	" " "
9271	Cooper's dip, 500 mg. Bluestone, 500 mg.	"	27.3.16 (killed)	" " "
8132	Cooper's dip, 500 mg. Bluestone, 500 mg.	"	31.3.16	Not a single H.C. Died as a sequel to haemonchosis.

Conclusions.—(1) The heavy wire-worm infection of the batch is proved by the cases of death before treatment and by the sequel of haemonchosis found on post-mortem examination of the treated sheep.

(2) The post-mortem examination on four treated sheep did not reveal a single H.C. in the abomasum, thus proving the complete effect of both mixtures used.

Experiment No. 29.—Twelve sheep aged from 4 to 6 years, in rather good condition, were infected for three days with larvae of H.C. mixed with bran. Some died on account of a gastro-enteritis, apparently of a specific nature, and some from haemonchosis, whilst the remainder were treated with sodium arsenite, 125 mg., in powder; bluestone, 500 mg., in powder, for two consecutive days.

The results are summarized in the following table:—

No. of Sheep.	Date of Infection.	Date of Treatment.	Date of Death.	Result.
8301	21/22.2.16	—	29.2.16 (killed)	Larvae of H.C. swarming on mucosa.
8322	"	—	5.3.16	No H.C.: enteritis.
9392	"	—	13.3.16	" "
8466	"	—	18.3.16	H.C. very numerous: acute anaemia.
8297	"	—	19.3.16	" "
7966	"	—	21.3.16	H.C. very numerous: acute anaemia and gastro-enteritis.
7713	—	23.3.16	7.4.16 (killed)	Not a single H.C.: anaemia.
8710	"	"	31.3.16 (killed)	" acute anaemia.
8204	—	"	1.4.16 (killed)	" "
8511	—	"	31.3.16 (killed)	" "
X	—	"	31.3.16 (killed)	" "
8199	—	"	31.3.16 (killed)	" "

Remarks.—(1) Seven days after infection the twelve sheep were looking dull, showing anorexia, diarrhoea, and temperatures ranging from 103° to 105.6° F. This fact, taken in connection with the six cases of death before treatment, indicates that the infection of H.C. was the cause of a specific gastro-enteritis to which three sheep (8322, 9392, 7966) succumbed. The absence of H.C. in the sheep Nos. 8322, 9392 can be explained by the assumption that the abnormal products of the abomasal contents killed the larvae of H.C. (2) The death of one sheep before treatment indicates the strong infection in the six sheep submitted to the treatment. (3) The treatment used was completely effective, as not a single worm was found in the treated sheep. (4) The sheep treated were very thin and anaemic on account of the heavy haemonchosis, but none of them showed ill effects after treatment.

Experiment No. 31 (29th April, 1916).—Twelve sheep, from 4 to 8 tooth, in fairly good condition, were infected with larvae mixed with bran for three consecutive days. On the 5th June, 1916, sheep No. 9398 was found *in extremis*, due to haemonchosis, and was killed. In the abomasum a mass of H.C., weighing 14 grammes, was found; some

of the worms were just commencing oviposition. The worms were counted, and found to comprise 5906 females, 2970 males, and 31 couples in copulation. Five other sheep were also poor, anaemic, dull looking, and were treated with sodium arsenite 125 mg. and bluestone 500 mg. for two consecutive days. The result is summarized in the following table:—

No. of Sheep.	Date of Treatment.	Date when Killed.	Result.
9238	6/7.6.16	19.6.16	Not a single H.C. in abomasum : poor.
9344	"	16.6.16	" " " marked anaemia.
9427	"	16.6.16	" " " poor.
X	"	—	Improving in condition : cultures negative.
Z	"	—	" " "

Remarks.—(1) Sheep No. 9344 when killed was *in extremis*, on account of acute anaemia as a sequel of haemonchosis. (2) Sheep No. 9238 and 9427 showed a decided improvement after treatment, and when killed were on the way to recovery. (3) Sheep X and Z were not killed as cultures, were negative, and they were kept as controls to see if recovery would be complete. Within the period of a month the conjunctiva was again pink in colour, and both sheep were in fairly good condition.

Experiment No. 51 (25th October, 1916).—Twelve sheep, rather poor in condition, due to the results of blue-tongue experiments a month previously, were infected with H.C. larvae by intra-rumenal injection of 10 c.c. watery suspension of larvae. At the time when the larvae were sheltered in the coagula of the abomasal-mucosa, the sheep were dosed with sodium arsenite, 125mg., in powder; bluestone, 500 mg., in powder. The results of the treatment given on 16th and 17th November, 1916, are given in the following table:—

No. of Sheep.	Date of Post-mortem Examination.	Result.
10528	13.11.16	Killed as a control—numerous thick coagula in abomasum with numerous larvae of H.C., young H.C. in 5th stage. Some were old.
10789	21.11.16	Not a single H.C. in abomasum : marked anaemia.
10629	"	" " "
10236	"	" " "
10693	"	" " "
10786	"	Five males of H.C. : marked anaemia.
10685	29.11.16	Four males of H.C. : marked anaemia.
10671	"	Not a single H.C. in abomasum : marked anaemia.
10725	"	" " "
10712	"	" " "
10732	"	" " "
10762	"	Eight females and fifteen males present in abomasum.

Remarks.—(1) The sheep killed for control purposes was selected as being in the best condition, and only showing a slight infection,

whereas the remainder had a heavy infection. The fact that thick coagula were found on post-mortem examination in the control sheep indicates that at the time of treatment the coagula present in the other sheep were even more numerous. (2) The lesions found in the abomasum of the five sheep killed three days after treatment were very striking, and showed that the infection of the batch before treatment must have been very heavy. (3) The sex of the worms found in the three sheep Nos. 10786, 10658, and 10762 supports the observations made in other cases, that the males of H.C. are more resistant than the females. (4) The treatment adopted in the above experiment was completely effective in eight cases out of eleven. In the other three cases it is probable that the few remaining worms were sheltered in some thick coagula, and consequently not reached by the medicine. (5) For the interpretation of the experiment it must be added: (a) Of the three negative cases one only would have practical importance in the field, as the two cases in which males of H.C. were found would not be carriers of infection. (b) Such cases of artificial infection are hardly possible in the field where the infection is not likely to be so sudden and so heavy, the consequence being that the coagula are not so thick or numerous, and the worms are easier to reach by treatment. (6) The case of sheep No. 9711, reported on fully in Experiment No. 35, can be referred to here as being of particular interest. It was infected two months previously, and was treated on the 8th and 9th September, 1916, with a double dose of sodium arsenite 100 mg., bluestone 500 mg. Four days after treatment it was killed, and the mucosa of the abomasum showed rather numerous blood coagula, but not a single adult or larvae of H.C. was found. This fact shows that the sheep had a natural recent infection following the artificial one, and that the treatment killed the worms of both infections.

Conclusions from Experiments Nos. 28 to 41.—(1) The dose of 125 mg. of sodium arsenite plus 500 mg. of bluestone in powder given for two consecutive days was well tolerated by adult sheep, and was completely effective for the wire-worm infection. (2) Sheep poor in condition and anaemic on account of haemonchosis, did not show any appreciable difference from the usual class of infected sheep in the resistance to the dose. (3) The exceptional cases in which the parasites resisted treatment do not appear to deserve special consideration, as the mode of infection under artificial and natural conditions differ widely.

(2) *Minimal effective dose of Sodium Arsenite plus Sulphate of Copper for two consecutive days*—*Summary of Experiments Nos. 33, 35, and 36.*—In Experiment No. 33 the dose of 100 mg. of sodium arsenite plus 500 mg. of bluestone was tested on four heavily infected sheep, and the dose of 100 mg. of sodium arsenite plus 400 mg. of bluestone was tested on four sheep also heavily infected. As the experiment proved successful, the treatment with the medicine in pills was attempted in Experiment No. 35, and the treatment again proved completely successful. Later, the above dose in powder was tested again in Experiment No. 65, and the effect on the wire-worm infection was completely effective.

Experiment No. 33.—Between the 23rd and 28th of June, twelve sheep from 4 to 8 tooth, in anaemic condition, were infected with

larvae of H.C. mixed with bran, when the degree of infection was demonstrated by two deaths from haemonchosis. The surviving sheep were treated. The dose given and the result is detailed in the following table:—

No. of Sheep.	Age of Sheep.	Date of Treatment.	Dose.	Date when Killed.	Date when Died.	Result.
X	4-tooth	—	—	—	July 15	Numerous H.C. just in fifth stage.
8456	8-tooth	—	—	—	Aug. 9	Numerous H.C. just in adult.
8364	4-tooth	Aug. 11, 12	Powder. 100 S.A., 500 B.S.	Aug. 23	—	Not a single H.C. in abomasum.
8664	6-tooth	"	"	" 24	—	" " "
9068	6-tooth	"	"	" 24	—	" " "
8489	4-tooth	"	"	" 21	—	" " "
8951	8-tooth	"	Powder. 100 S.A., 400 B.S.	" 22	—	Three males of H.C. in abomasum.
8947	6-tooth	"	"	" 23	—	Not a single H.C. in abomasum.
9418	6-tooth	"	"	" 25	—	" " "
8943	4-tooth	"	"	" 26	—	" " "
8481	6-tooth	Control sheep	—	" 26	—	Very numerous H.C. in abomasum.
9435	6-tooth	" "	—	" 26	—	200 H.C. in abomasum.

Remarks.—The heavy infection of the sheep is proved by the deaths of two sheep from haemonchosis before treatment, and the post-mortem examination on the two sheep killed without treatment.

Conclusions.—(1) The dose of 100 mg. of sodium arsenite, plus 500 mg. of bluestone in powder, was completely effective. (2) The dose of 100 mg. of sodium arsenite, plus 400 mg. of bluestone in powder, was effective, with the exception of one treated sheep, in which three male H.C. were found. (3) A control of the result of infection was also made by cultures before and after treatment. It was, however, found that a month after infection, when it could be expected that worms were in the oviposition period, the result of the cultures was not in constant proportion to the actual infection, and on some days the larvae were even very scarce. Eight days after treatment it was found that larvae of H.C., or those very much resembling them, could still be found in cultures of faeces, thus indicating the difficulties met with when drawing conclusions from cultural tests alone.

Experiment No. 35.—On the 5th, 6th, 7th, and 8th July, 1916, seventeen sheep just discharged from blue-tongue vaccine preparation, were infected with larvae of H.C. mixed with bran, and one was killed later to test the result of infection. This sheep, which was in poor condition, was found on post-mortem examination to be infected, but of the remaining sixteen sheep ten did not present clinical symptoms. One of the remaining six was dosed with the medicine in powder, and the other five were dosed with the medicine in pill-form. The result of treatment was as follows:—

No. of Sheep.	Age of Sheep.	Date of Treatment.	Dose.	Date when Killed.	Result.
9693	8-tooth	—	—	Aug. 28	Very numerous H.C. in abomasum.
9702	6-tooth	Aug. 29/30	S.A. B.S. Powder 100 + 500 Pill	" 31	Not a single H.C. in abomasum.
9740	4-tooth	Sept. 8/9	100 + 500	Sept. 11	" " "
9711	6-tooth	—	"	" 13	" " "
9732	8-tooth	—	"	" 13	" " "
9694	2-tooth	—	"	" 28	" " "
9731	8-tooth	—	"	" 28	Four males and five females of H.C. in abomasum.

Remarks.—(1) Sheep No. 9702 when dosed was in extreme condition of anaemia, very thin, with typical "bottle-jaw," unable to stand on account of weakness, showed diarrhoea, and the faeces were very rich in eggs. (The case of this particular sheep was considered to be one of the most severe infections met with.) (2) Two hours after the administration of the second dose the lips of sheep No. 9731 were of a greenish colour, and small pieces of the pill were adherent in the hairs on the lips, thus showing that the pill had broken up in the mouth and pieces had been ejected. (3) Sheep Nos. 9711 and 9732 showed small coagula mixed with mucus adhering to the abomasal mucosa, but no larvae or worms were found in their coagula. Seeing that two months elapsed since artificial infection the presence of the coagula could only be explained by a recent infection with larvae picked up in the stable. It also appeared that the larvae in their coagula were killed by the treatment.

Conclusions.—(1) The above treatment was supported by sheep that were in the extreme stages of haemonchosis. (2) The treatment with pills was as effective as the treatment with powder. (3) The treatment was effective both with adult worms and with larvae in coagula. (4) The presence of a few H.C. in sheep No. 9731 is explained by the incomplete dose and shows that pills are more liable to be ejected by sheep than the powder.

Experiment No. 65.—On the 11th April, 1917, ten sheep in various conditions of nutrition were heavily infected through the rumen with H.C. In the months following the infection three sheep were killed (see also Experiment No. 58).

A month and a half after infection the remainder of the sheep were treated with sodium arsenite 100 mg. and bluestone 500 mg. in powder for two consecutive days. The result is recorded in the following table:—

No. of Sheep Killed before Treatment.	No. of Sheep Killed after Treatment on 27/28.5.17	Date when Killed.	Result.
9423	—	5.5.17	Small number of H.C. in abomasum.
10683	—	21.5.17	" " "
11387	—	22.5.17	Very numerous H.C. in abomasum.
—	10783	2.6.17	Not a single H.C. in abomasum.
—	11328	2.6.17	" "

Remarks.—From the clinical appearance of the infected sheep and from the post-mortem result of the control sheep it must be concluded that the infection in the sheep was not homogeneous, and in some was not heavy.

Conclusions.—The result of treatment was completely successful.

Conclusions on the Minimal Effective Dose.—(1) The dose of 100 mg. of sodium arsenite, plus 500 mg. of bluestone, was completely effective on heavily infected sheep. (2) The above dose was provisionally called "The Minimal Effective Dose."

(3) *Maximal Safe Dose of Sodium Arsenite, plus Bluestone, given for two consecutive days.*—*Summary of Experiments Nos. 50, 50A, and 63.*—The two first experiments of this series deal only with the resistance of the sheep to the dose as the effect on the worms must a priori be complete. In Experiment No. 50 the dose of 200 mg. of sodium arsenite, plus 500 mg. of bluestone, was tested. The mortality from arsenical poisoning was 40 per cent., but there was also very strong grounds for suspecting the hot weather prevailing at the time as being a complicatory factor. Nevertheless, to be on the safe side, in Experiment No. 50A the dose of arsenite was reduced to 150 mg. and no deaths occurred in the dosed sheep.

Experiment No. 63 was undertaken for other purposes, but as the resistance of the sheep to the dose of 150 mg. of sodium arsenite, and the effect on the worms is well brought out, it was thought interesting to record it here. According to the result of the above experiments it was concluded that as far as laboratory experiments were concerned the dose of 150 mg. of sodium arsenite should be considered the maximal safe dose, and the dose of 200 mg. should tentatively be considered the minimal fatal dose.

Details of Experiments.—*Experiment No. 50.*—Full-grown sheep Nos. 9786, 10066, 9995, 9752, and 9713, good condition, weighing from 45 lb. to 80 lb., were dosed on the 23rd and 24th November, 1916, with sodium arsenite 200 mg. in powder and bluestone 500 mg. in powder. The usual dietetic method was followed. (25th November, 1916.) Sheep No. 9752, weight 70 lb., died of acute arsenical poisoning. (26th November, 1916.) Sheep No. 9786, weight 75 lb., died from acute arsenical poisoning.

Remarks.—During treatment the temperature in the stable where the sheep were dosed ranged from 90° F. to 110° F. On the 14th November, 1916, sheep No. 10018, in good condition and belonging to the same lot, died from heat stroke.

Conclusions.—It is presumed that the death from arsenical poisoning of the above two sheep was aggravated by the exceptionally high outside temperature, but in all probability similar conditions of temperature would be met with in the practice. The dose of 200 mg. of sodium arsenite, plus 500 mg. of bluestone, must be considered as dangerous under peculiar conditions of climate.

Experiment No. 50A.—Sheep Nos. 9724, weighing 75 lb.; 9753, 90 lb.; 9714, 90 lb.; 9705, 100 lb., belonging to the same lot of sheep as in Experiment 50, and in good condition, were dosed on the 1st and 2nd December, 1916, with sodium arsenite 150 mg. and bluestone 500 mg. in powder form. During and after dosing the temperature remained very high. The sheep were kept under observation in the stable for twelve days, but no ill effects were noted.

Experiment No. 63.—Seven adult sheep were heavily infected with H.C. larvae between the 26th February, 1917, and 2nd March, 1917, and four of them were dosed on the 27th and 28th March, 1917, with sodium arsenite 150 mg. and bluestone 500 mg. in powder form. The other three sheep were kept for controls. The four sheep showed slight anorexia and dullness after dosing, but on the night of the 28th they were all ruminating. The result is reported in the following table:—

No. of Sheep.	Age of Sheep.	Weight of Sheep.	Date when Killed.	Condition of Abomasum.	Result.
11335	Treated 8-tooth	sheep. 50 lb.	3.4.17	Pale	Not a single H.C. <i>Oes. col.</i> were present.
11331	6-tooth	68 lb.	—	—	Cultures; some rhabditis and <i>Strong</i> larvae.
11343	8-tooth	75 lb.	2.4.17	Pale	Not a single H.C. <i>Oes. col.</i> frequent.
11340	6-tooth	60 lb.	—	—	Cultures: some rhabditis and <i>Strong</i> larvae.
11369	Control sheep. 8-tooth	70 lb.	—	—	Cultures from faeces very rich in H.C. larvae.
11387	8-tooth	80 lb.	—	—	Cultures from faeces very rich in H.C. larvae.
11321	8-tooth	75 lb.	30.3.17 (died)	Pale O. dematous	Abomasum swarming with H.C.

Remarks.—(1) The few larvae of the *Strongylus* type found in the cultures of sheep Nos. 11331 and 11340 were in all probability from some *Oes. col.* located in the large intestine similarly to what was noted in the post-mortem examination of Nos. 11335 and 11343. (2) No. 11321 was not killed, but died suddenly of extreme haemonchosis on the 30th March, 1917. (3) Sheep Nos. 11369 and 11387 showed clinically a very heavy infection and were not killed.

Conclusion.—The dose of 150 mg. of sodium arsenite, plus 500 mg. of bluestone was completely effective, and no ill effects or lesions in the digestive organs were noted in the treated sheep. Other experiments proving the safety of the "maximal safe dose" of sodium arsenite, plus bluestone, are summarized in the following table, and some of them will be reported more extensively later, under the special headings to which they refer.

No. of Experiment.	Date of Experiment.	Number of Sheep Treated.	State of Drugs Used.	Result.
45	19.12.16	{ 390 5 goats }	Clear solution ...	Satisfactory.
56	20.12.16	5	Powder ...	"
		5	Turbid solution ...	"
		5	Clear solution ...	"
57A	21.12.16	43	Powder ...	"
		25	Turbid solution ...	"
		25	Clear solution ...	"
57B	1.2.17	100	Powder ...	"
		132	Clear solution ...	"
57C	8.2.17	124	Clear solution ...	"
57D	12.2.17	125	In powder ...	One sheep dead.

Remarks.—In Experiment No. 57D one sheep was found dead in the morning of 15th February, 1917. The hairs round the lips contained clusters of moist green powder amounting to about half a single dose of the medicine. Post-mortem examination showed that the cause of death was probably arsenical poisoning, and as the second dose of powder was only partially swallowed it shows that the sheep was apparently poisoned by the first dose. This fact allows of the conclusion that the sheep had an exceptionally high susceptibility to arsenic.

Conclusions on the Maximal Safe Dose.—(1) The dose of 200 mg. of sodium arsenite, plus 500 mg. of bluestone, was found fatal in 40 per cent. of treated sheep, and was considered to be a dose above the limit of safety. (2) The dose of 150 mg. of sodium arsenite, plus 500 mg. bluestone, was found completely safe, and was considered as the maximal dose below the limit of safety.

(d) *Treatment of Lambs 9 to 10 months old with Sodium Arsenite and Bluestone.*

Summary of Experiments Nos. 32, 47, 49, 37, and 44.—For these experiments lambs from 6 to 10 months old were preferably used, as at this age the lamb is strong enough to stand a heavy infection. As the average weight of a lamb of 6 to 10 months is about three-fifths the weight of the full-grown sheep, in Experiment No. 32 some heavily infected lambs 9 to 10 months old were treated with three-fifths of the full dose, viz., 75 mg. of sodium arsenite, plus 300 mg. of bluestone. The general dietetic treatment adopted was similar to that for full-grown sheep. In the same experiment some lambs of the same age were dosed with 75 mg. of sodium arsenite alone with the object of ascertaining whether bluestone is an indispensable ingredient in the composition of the dose. The experiment proved that a mixture of bluestone, plus sodium arsenite, was more effective than sodium arsenite alone.

Brief results of other experiments proving the success of the three-fifths dose are recorded here, but details of them reported later in Experiments Nos. 47 and 49 (Comparative Experiments on the Therapeutic Value of some of the more Common Drugs against Wire-worm Infection).

In Experiment No. 37, dealing with the monthly treatment of sheep, which is fully reported later, some observations are recorded showing that 90 mg. sodium arsenite, plus 300 mg. bluestone, is the maximal safe dose for lambs 9 to 10 months old.

Experiment No. 44 was undertaken for the purpose of seeing whether the average dose for lambs 10 months old is effective when H.C. are sheltered in the coagula. The result was completely successful in two heavily infected lambs.

Details of Experiments.—*Experiment No. 32.*—On the 15th, 16th, and 17th of May, 1916, seven lambs 7 to 8 months old were heavily infected with larvae of H.C. mixed with bran.

On the 6th and 7th of July, when the infection of the lambs was proved by their anaemic condition and by the rich colony of larvae found in cultures of faeces, the following treatment was undertaken:—Two lambs were dosed with 75 mg. of sodium arsenite, plus 300 mg.

of bluestone, and three lambs were dosed with 75 mg. of sodium arsenite. The result is given in the following table:—

Lambs Dosed with Sodium Arsenite plus Bluestone.	Lambs Dosed with Sodium Arsenite alone.	Date when Killed.	Result.
9591	—	22.7.16	Not a single H.C. Lamb pale; hydraemic.
9533	—	25.7.16	—
—	9514	17.7.16	293 H.C. in abomasum; anaemic.
—	9582	24.7.16	Numerous H.C. in abomasum; anaemic.
—	9519	1.8.16	Not a single H.C.; anaemic; hydraemic.

Remarks.—No ill effects were noted in the lambs as a result of treatment.

Conclusions.—(1) The dose of 75 mg. of sodium arsenite, plus 300 mg. of bluestone, was well tolerated by anaemic lambs 9 to 10 months old. (2) The effect on the wire-worms was complete. (3) The dose of 75 mg. of sodium arsenite alone was well tolerated by the lambs, but was insufficient for destroying the worms.

Experiment No. 47.—Two heavily infected lambs 9 to 10 months old, weighing from 42 lb. to 43 lb., were treated with the double dose of 75 mg. of sodium arsenite, plus 300 mg. of bluestone, and were killed some days later. The post-mortem examination showed the typical lesions of haemonchosis, but not a single H.C. was found in the abomasum. For further details see "Comparative Experiments on the Therapeutic Value of some of the more Common Drugs against Wire-worm Infection."

Experiment No. 49.—Five lambs from 10 to 11 months old, weighing about 45 lb. and heavily infected with wire-worms, were treated with the maximal safe dose. The lambs were all killed afterwards, when the typical lesions of haemonchosis were found, but not a single H.C. was detected in the abomasum. For fuller details see "Comparative Effects of Sodium Arsenite, plus Bluestone, with other well-known Anthelmintics."

Experiment No. 37.—The experiment was made on fifty-three sheep, of which thirty-three were just showing the two middle teeth (12 months old) and twenty were lambs from 9 to 10 months old. The average weight of the batch was 48 lb. per head. (5th December, 1916.) 9 a.m.: lambs dosed with sodium arsenite 90 mg. and bluestone 300 mg., dissolved in 6 c.c. of water. 2 p.m.: one lamb (No. 9514) not eating; the rest did not show any ill effects. (6th December, 1916.) 2 p.m.: one lamb (No. 9596) not eating. No ill effects were noted in the above lot of lambs.

Experiment No. 44.—On the 7th, 8th, and 9th of September, 1916, lambs Nos. 9515 and 9518, both 11 months old, were heavily infected with larvae mixed with bran. On the 17th September, 1916, No. 9518 showed diarrhoea. Both lambs were ill. On the 19th and 20th the lambs were dosed with sodium arsenite 75 mg., bluestone 300 mg. in pill form. (27th September, 1916.) No. 9518 still showed marked anaemia and diarrhoea and was killed. In the abomasum not a single larvae or adult H.C. was found. Remnants of blood coagula were still scattered on the mucosa. (27th September, 1916.) No. 9515

was slightly anaemic and was killed. In the abomasum not a single H.C. was found. The mucosa showed catarrhal hyperaemia and small old remnants of blood coagula mixed with mucus.

Conclusions.—The average dose for lambs of 10 months was completely effective in two heavily infected lambs, when the H.C. were sheltered in the coagula.

(e) Treatment of Lambs under 9 months old with Sodium Arsenite and Bluestone.

Summary of Experiments Nos. 41, 46, 46A, 77, 67, and 76.—In Experiment No. 41 the "average dose for lambs 10 months old" was tested on lambs 3 months old, but did not prove to be safe. In Experiments Nos. 46 and 46A the dose of 50 mg. of sodium arsenite, plus 300 mg. of bluestone, was tested on lambs 4 to 5 months old, but the result was not reliable owing to the lambs being ill-conditioned. Experiments Nos. 77, 67, and 76 were undertaken on private farms on lambs from 3 to 6 months old, and no symptoms of illness followed.

Details of Experiments.—*Experiment No. 41.*—On the 22nd August, 1916, six lambs of about 3 months old, and weighing from 23 lb. to 26 lb., were dosed for two consecutive days with sodium arsenite 75 mg. and bluestone 300 mg. in powder form. One lamb showed dullness and anorexia for three days after treatment. A second lamb showed colic and anorexia on the day following the second dose, appeared to be healthy for the next fourteen days, but died after a day's illness, during which the neck was bent to one side with the nose at the level of the coastal region, and there was a paralysis of the mouth. The post-mortem examination did not show anything abnormal. The mouth and pharynx were full of unmasticated grass.

Conclusions.—The result of the experiment shows that the above dose could not be used on small lambs with safety.

Experiment No. 46.—On the 4th October, 1916, ten lambs from 4 to 5 months old, some of which were weak, were infected with an injection of 10 c.c. of a watery suspension of larvae into the rumen. On the 12th October, 1916, five lambs were treated with sodium arsenite 50 mg. and bluestone 599 mg. in powder form. The result of the experiment is summarized in the following table:—

Lamb.	Weight in Pounds.	Treated or Control.	Date when Died.	Date when Killed	Result of Post-mortem Examination.
1	26	Control	—	9.10.16	Numerous H.C. larvae in abomasum under a thick coat of catarrh.
2	16	Treated	14.10.16	—	Died of arsenical poisoning. No H.C. in abomasum.
3	28	Control	16.10.16	—	Died of heavy haemonchosis.
4	25	Treated	16.10.16	—	" "
5	30	Control	17.10.16	—	" "
6	29	"	20.10.16	—	" "
7	32	"	22.10.16	—	" "
8	35	Treated	—	31.10.16	Not a "single H.C. Some Cooperia pectinata.
9	30	"	—	"	" "
10	32	"	—	"	H.C. old and of recent infection fairly numerous.

Remarks.—(1) Lamb No. 2 showed at post-mortem examination marked hyperaemia of the sulcus omasi, haemorrhagic infiltration in the cardiac region of the abomasal mucosa, hyperaemia and oedema of lungs, and hyperaemia of kidneys. (2) Lamb No. 4 showed at the post-mortem examination an impaction of the rumen and omasum. The greenish colour of the bluestone and arsenic was easily detected on the hard mass of the contents in the anterior dorsal blind sac of the rumen. The abomasum contained a big semi-solid coagulum of blood mixed with mucus, in which H.C. in the fourth and fifth stages were exceedingly numerous. The mucosa of the abomasum was very pale with numerous petechiae. It appears that the primary cause of death was H.C. infection, which produced a heavy haemorrhagia in the abomasum. The impaction of the rumen and abomasum was apparently secondary to the parasitic gastritis. In this case the medicine passed into the rumen and did not reach the abomasum. (3) Nos. 5 and 6 showed on post-mortem examination the same condition of the abomasum as No. 4. (4) Nos. 8, 9, and 10 were also very anaemic and showed hydraemia in the internal organs. No. 10 showed a very thick and firm coat of catarrh on the abomasal mucosa, under which the worms were sheltered.

Conclusions.—(1) In the above experiment the injection of 10 c.c. of a watery suspension of larvae into the rumen gave two strong infections in lambs, and haemonchosis was allowed to progress too far before treatment was attempted. (2) The number of lambs that died without being dosed showed that at the time of treatment there was but little hope of saving them. The thick coat of blood and catarrh found on the abomasal mucosa was, in the majority of cases, far beyond any stage met with in cases of natural infection, thus giving the worms an unusually advantageous shelter from the action of the medicine. (3) The case of poisoning in lamb No. 2 can be easily explained by the extremely grave parasitic gastritis at the time of dosing. The death of lamb No. 4, which was also treated, was purely due to haemonchosis, and apparently no worm was killed by the treatment as the medicine did not reach the abomasum.

Experiment No. 46A.—On the 7th, 8th, and 9th of August, 1916, two lambs, 4 months old, were infected with larvae mixed with bran. On the 19th September, 1916, lamb A was pale and dull with strong diarrhoea. On the 19th and 20th of August the two lambs were dosed with a pill consisting of sodium arsenite 50 mg. and bluestone 300 mg. lamb A died in the afternoon of the 20th. The alimentary canal and the other internal organs did not show any symptoms of poisoning. The main features on post-mortem examination were anaemia and hydraemia. No H.C. were found in the abomasum. On the 22nd lamb B, not showing any symptoms of poisoning, was killed. Very marked anaemia and hydraemia were noted at post-mortem examination. The abomasum was distended and contained a thick mass of coagulated blood weighing 40 grammes. The abomasal mucosa was swarming with H.C. that had just reached the adult stage.

Remarks.—(1) Lamb A did not die from arsenical poisoning, but from haemonchosis. The complete absence of H.C. in the abomasum could be explained on the assumption that toxic gastritis killed the H.C. before the death of the host and were carried out by the faeces

The same observation was frequently made on sheep that were artificially infected and showed typical acute haemonchosis. On post-mortem examination a marked gastritis was noted, but there were no H.C. in the abomasum. (2) The result of the post-mortem examination on lamb B gave strong grounds for the belief that by reason of the blood impaction in the abomasum produced by the H.C., nothing passed from the rumen to the abomasum for some days, and accordingly the medicine did not come into contact with the worms.

Conclusions.—In the above experiments the lambs were on the point of death, and the condition of the infection was so remote from what happens under natural conditions that they were unsuitable for treatment.

Experiment No. 77.—This experiment will be fully reported in the paragraph dealing with the "Treatment applied on the Farms." It need only be mentioned here that four cross-bred lambs, 4 months old, were treated for two days with sodium arsenite 50 mg. and bluestone 300 mg. The lambs were slightly cut up in condition on the day of the second dose, but no other symptoms of illness were observed.

Experiment No. 67.—Full reference to this experiment is also given in the paragraphs dealing with the "Treatment applied on the Farms." In this experiment three lambs, 2 months old, were dosed for two days with sodium arsenite 35 mg. and bluestone 140 mg. Nine other lambs, 4 months old, were dosed for two days with sodium arsenite 50 mg. and bluestone 200 mg. No symptoms of illness were noted.

Experiment No. 76.—This experiment will also be reported in full in paragraphs dealing with the "Influence of the Weather on Treatment." In this experiment nineteen Merino lambs about 6 months old were dosed for two days with sodium arsenite 75 mg. and bluestone 300 mg. No symptoms of illness were observed.

General Conclusions on the Results of the Treatment on Lambs.

1. The safe dose for lambs of 6 to 10 months is—
Sodium arsenite, 75 mg.; bluestone, 300 mg.
2. The safe dose for lambs of 4 to 6 months is—
Sodium arsenite, 50 mg.; bluestone, 200 mg.
3. The safe dose for lambs of 2 to 4 months is—
Sodium arsenite, 35 mg.; bluestone, 140 mg.

(f) Treatment of Pregnant Ewes.

Three experiments, undertaken primarily with another object, can be referred to briefly here, as the question of the treatment of pregnant ewes is an important one for the sheep farmer. In the two first experiments (Nos. 42 and 43), pregnant ewes, just discharged from blue-tongue experiments, were dosed. Several of them lambed soon afterwards, and in three out of twelve cases the lambs died within two days of birth. In one case a ewe died of difficult parturition. Such mortality is often observed amongst pregnant ewes after bleeding for a vaccine, and in the case of the ewes referred to in the present experiment it is difficult to know whether to ascribe the cause of the

mortality to blue-tongue sequel or to dosing with bluestone and sodium arsenite. However, in the third experiment (No. 67, reported fully under the heading of "Treatment applied on the Farms") 108 pregnant ewes were treated with a larger dose of bluestone and sodium arsenite than that found to be safe, and yet no mortality whatever occurred. It would, therefore, appear that the dosing of pregnant ewes could be undertaken without danger, but more work is necessary before making a definite conclusion.

D.—FORM OF THE MEDICINE.

(a) *Summary of Experiments—Powder Form.*—In the initial experiments the drugs were administered in powder form, and good results were usually obtained. It was, however, observed that about 10 per cent. of the sheep ejected part of the powder when moistened with saliva, and it was thought that the powder form would perhaps not be the most convenient for the farmer.

(b) *Pill Form.*—Accordingly, attempts were made to administer the dose in pill form. A number of experiments with stained pills have been recorded in the paragraph dealing with the "Passage of the Drugs," and it was found that in one case out of nineteen the pill was ejected after having been retained for some time in the pharynx (sheep No. 9668). It has been seen in Experiment No. 35 (reported in full in the paragraph dealing with the minimal effective dose) that out of five sheep dosed with the pill containing the minimal effective dose, four did not show a single H.C. in the abomasum, whilst the fifth showed nine, and in the conclusions of this experiment the explanation of the incomplete success was that this particular sheep ejected part of the pill after breaking it in the mouth. In Experiment No. 44 (see treatment of lambs under 9 months old) the result of the medicine in pill was completely successful. In Experiment No. 46, the result with the medicine in pill form was not successful, owing chiefly to the far advanced state of infection. No further experiments were carried out with the dose in pill form, as it was thought that the pill is more liable to be broken up and ejected, or to be retained for some time in the pharynx than the powder.

(c) *Liquid Form.*—Dealing with experiments with the medicine in solution, the first purpose was to reduce the bulk of water to a minimum, as it is well known that the greater the amount of liquid the greater probability of its entering the trachea. Consequently the average dose was dissolved in 10 c.c. of water, with the addition of a small amount of hydrochloric acid. The solution was administered by means of a syringe of 10 c.c. capacity, and this was found to be an easy method, and enabled the dose to be accurately measured. In Experiment No. 55 the average dose of sodium arsenite plus bluestone was administered in liquid form, and the result was not so satisfactory as in the powder form. In Experiment No. 56 the maximal safe dose

of sodium arsenite plus bluestone was administered in liquid form and the result was completely successful. In Experiment No. 66, a comparative experiment on the effect of the average dose in powder and in liquid form was made as a control to Experiment No. 35. In this case the liquid dose was as effective as the powder. This experiment gives grounds for concluding that for practical purposes on a farm the average dose can be used equally well either in liquid or powder form. It can be mentioned that the maximal dose in liquid form is a priori less harmful for the abomasal mucosa than the powder form, as no caustic action is produced by lumps of moist powder being brought into contact with the mucosa of the stomach. Further, it was already reported in the paragraph on the "maximal safe dose" that in Experiments Nos. 45, 56, 57A, 57B, and 57C, the resistance of the sheep to the maximal safe dose in liquid form (turbid and clear) was quite satisfactory.

Details of Experiments.—*Experiment No. 55.*—On the 28th October, 1916, eleven sheep in an anaemic state were infected with 10 c.c. of suspension of H.C. larvae in water through the rumen. One was killed on the 15th November, 1916, to ascertain the conditions of infection. On the 29th and 30th of November, when the sheep proved to be infected with haemonchosis, ten of them were treated with sodium arsenite, 125 mg.; bluestone, 500 mg.; hydrochloric acid, 0.15 c.c.; water, 10 c.c. In six of them the liquid was injected into the mouth with a Pravaz's syringe, in two of them the liquid was injected directly into the rumen through the left flank, whilst the remaining two were kept as controls. The results of the experiments are summarized in the following table:—

Sheep Dosed on 29th and 30th November, 1916.		Control Sheep.	Date when Killed.	Results.
Through the Mouth.	Through the Rumen.			
—	—	10435	15.11.16	Numerous adult H.C. and numerous coagula with larvae of H.C.
10567	—	—	6.12.16	Not a single H.C.
10668	—	—	6.12.16	Sheep pale.
10703	—	—	14.12.16	Sheep pale and hydraemic.
10736	—	—	14.12.16	" "
10660	—	—	7.12.16	Eight females and ten males of H.C. in abomasum.
10771	—	—	14.12.16	Four females and fifteen males of H.C. in abomasum.
—	10705	—	14.12.16	Fairly numerous H.C. in abomasum.
—	10679	—	—	Not killed; culture of faeces shows larvae of H.C.
—	—	10714	6.12.16	Numerous H.C. in abomasum.
—	—	10769	—	Not killed; culture of faeces shows larvae of H.C.

Conclusions.—(1) The solution of 125 mg. of sodium arsenite, plus 500 mg. of bluestone, in watery solution failed to kill all worms in two cases out of six. (2) The same strength of solution injected through the flank into the rumen failed in both cases.

Experiment No. 56.—Twenty-one sheep were infected on the 25th November, 1916, through the rumen with larvae of H.C. About a month after all sheep showed haemonchosis, whilst two of them died. Of the surviving nineteen, fifteen were treated with the following doses on the 20th and 21st December, whilst the remaining four were kept as controls:—

First Lot of Five.—Sodium arsenite 150 mg., bluestone 500 mg., water 10 c.c., without the addition of any hydrochloric acid (turbid solution).

Second Lot of Five.—Sodium arsenite 150 mg., bluestone 500 mg., hydrochloric acid 0.15 c.c., water 10 c.c. (clear solution).

Third Lot of Five.—Sodium arsenite 150 mg., bluestone 500 mg. (in powder).

The result of the experiment is summarized in the following table:—

Sheep Dosed with Clear Solution.	Sheep Dosed with Turbid Solution.	Sheep Dosed with Powder.	Control Sheep.	Date when Killed.	Result.
—	—	—	10825	15.12.16	Sheep died of acute haemonchosis.
—	—	—	10880	19.12.16	Very numerous H.C. in abomasum.
—	10905	—	—	28.12.16	" " " "
—	10800	—	—	28.12.16	Not a single H.C. Marked anaemia.
—	10797	—	—	3.1.17	" " " "
—	9707	—	—	6.1.17	" " " "
—	10897	—	—	28.2.17	Six males H.C. Marked anaemia.
8432	—	—	—	28.12.16	Not a single H.C. Anaemia and hydraemia
10568	—	—	—	28.12.16	" " " "
10822	—	—	—	29.12.16	Not a single H.C. Anaemia and marasmus.
10810	—	—	—	3.1.17	" " " "
10649	—	—	—	6.1.17	Not a single H.C. Marked anaemia.
—	—	10969	—	28.12.16	Not a single H.C. Anaemia and marasmus.
—	—	10888	—	28.12.16	" " " "
—	—	10884	—	29.12.16	" " " "
—	—	9722	—	3.1.17	Not a single H.C. Marked anaemia.
—	—	10713	—	6.1.17	" " " " Anaemia.
—	—	—	10768	29.12.16	Very numerous H.C. Anaemia.
—	—	—	9723	3.1.17	Very numerous H.C. from artificial infection. Fairly numerous H.C. of a previous natural infection.
—	—	—	10907	6.1.17	" " " "
—	—	—	9725	—	Clinical symptoms of haemonchosis on 28.12.16, but not killed.

Remarks.—(1) The turbid solution could be more accurately called a suspension, as the solution was incomplete and the undissolved compounds formed a deposit, rendering correct dosing almost impossible. This must be the explanation for the presence of the six H.C. which resisted treatment in sheep No. 10897. (2) The two

sheep which died of of haemonchosis before treatment, the marked anaemia and hydraemia in the treated sheep, and the very numerous H.C. found at the post-mortem of the four sheep killed for control purposes, showed that the treated sheep were heavily infected with wire-worms.

Experiment No. 66.—On the 19th May, 1917, eight full-mouth sheep, proved by culture and clinical symptoms to be infected, were divided into two lots. One lot was dosed for two consecutive days with sodium arsenite 125 mg. and bluestone 500 mg. in powder. The other lot was dosed for two consecutive days with sodium arsenite 125 mg., bluestone 500 mg., water 10 c.c., hydrochloric acid 0.15 c.c. The result of the experiment was as follows:—

Sheep Dosed with Powder.	Sheep Dosed with Liquid.	Date when Killed.	Result.
11098	—	26.5.17	Not a single H.C. Mucosa abomasum pale.
11455	—	28.5.17	Not killed; cultures of faeces negative.
11177; 11351	—	—	Not a single H.C. Anaemia and hydraemia.
—	11354	26.5.17	Not killed; cultures of faeces negative.
—	11461	28.5.17	Not killed; cultures of faeces negative.
—	11418; 11476	—	Not killed; cultures of faeces negative.

General Conclusions on the form of the Medicine.

(1) The powder form was the most effective method, providing the drugs actually passed into the stomach. (2) The pill form would seem to be more convenient for farmers, but it has the objection that it gives the sheep a better chance of ejecting fragments after the pill has been broken up in the mouth. (3) Administering the drugs in the liquid form appears to overcome both difficulties. (4) The administration of the maximal safe dose of sodium arsenite (150 mg.) and bluestone (500 mg.) in liquid form was completely effective. (5) The addition of hydrochloric acid to the liquid was a distinct improvement and ensured a complete solution. (6) The maximal safe dose in liquid form has an additional advantage on the powder form in so far as it is better supported by the mucosa of the abomasum and reduces the possibility of overdosing.

E.—DIETETIC REQUIREMENTS DURING TREATMENT.

Summary of Experiments Nos. 52, 53, 75A, and 75B.

In Experiment No. 52 sheep were allowed to feed during the two days of treatment, but no water was given. The result was completely effective on the worms and well supported by the sheep. In Experiment No. 53 the dosed sheep were allowed free access to food and water,

but the result showed that the treatment was dangerous for the sheep and interfered with the effect of the drugs. Experiment No. 75A was carried out to see whether an addition of common salt to the average dose (sodium arsenite and bluestone) could be made with safety for the sheep, whilst Experiment No. 75B had the object of ascertaining whether sheep could be allowed free access to a lick.

Details of Experiments.—Experiment No. 52.—On the 25th October, 1916, twelve sheep, in fair condition, were injected with 10 c.c. of a watery suspension of H.C. larvae, with positive results. On the 16th and 17th November, 1916, five of the sheep were dosed with sodium arsenite 125 mg. and bluestone 500 mg. powder. They were kept without water; food was given between 2 to 4 p.m. each day. At the same time six other sheep were dosed with the same quantity. During the two days of treatment they were allowed to eat freely, but water was not allowed. The result of the experiment is summarized in the following table:—

Sheep Treated.		Date when Killed.	Result.
Starved.	Not Starved.		
10723	—	Not killed	—
10675	—	Not killed	—
10698	—	4.12.16	Not a single H.C. in abomasum.
10729	—	Not killed	—
10635	—	4.12.16	Not a single H.C. in abomasum.
—	10634	Not killed	—
—	10753	Not killed	—
—	10661	4.12.16	Not a single H.C. in abomasum.
—	10776	Not killed	—
—	10574	4.12.16	Not a single H.C. in abomasum.
—	10702	Not killed	—
—	10677	14.11.16	Killed for control.

Remarks.—(1) On the 14th November, 1916, Sheep No. 10677, killed for control to prove the infection, was the one in the best condition, and on the same day the other eleven sheep were anaemic and in poor condition. (2) The four sheep killed after treatment were those that showed more marked symptoms of haemonchosis. (3) No ill effects were noted in the treated sheep.

Experiment No. 53 (25th October, 1916).—Twelve sheep used one month previously for blue-tongue vaccine were infected by injecting into the mouth 10 c.c. of a watery suspension of H.C. larvae. On the 14th November, 1916, the sheep were looking rather poor and very pale, showing a very heavy haemonchosis. On the 17th and 18th November, 1916, eleven of them were dosed with sodium arsenite 125 mg. and bluestone 500 mg. in powder. The sheep were kept in a stable, bedded on hay, and allowed free access to water, and were fed daily at 7 a.m. and at 4 p.m. with green grass. During the two days

of treatment the sheep ate as usual. The result of the experiment is summarized in the following table:—

No. of Sheep.	Date when Killed.	Result.
10671	14.11.16	Killed for control. Very numerous H.C. in abomasum.
10755	22.11.16 (died)	Ulcerative enteritis. No H.C. in abomasum. Arsenical poisoning.
10643	22.11.16	Fair amount of H.C. in abomasum.
10777	"	Very numerous H.C. in abomasum.
10329	"	" "
10565	"	Fair amount of H.C. in abomasum.
10708	"	" "

Remarks.—The remaining four sheep were not killed, as cultures made from faeces on the 22nd November, 1916, were all positive.

Experiment No. 75A.—On the 23rd and 24th June, 1917, sixteen adult sheep were dosed with sodium arsenite 125 mg. and bluestone 500 mg. in powder, plus a teaspoonful (5 gm.) of common powdered salt. On the morning of the 25th June, 1917, one sheep was found dead, and on post-mortem examination showed haemorrhagic gastritis, hyperaemia of the small intestines, haemorrhagic typhlitis, hyperaemia of kidneys, fatty degeneration of liver, hyperaemia and oedema of lungs. The cause of death was ascribed to arsenical poisoning.

Experiment No. 76B.—On the 26th and 27th June, 1917, twenty-four sheep were dosed with sodium arsenite 125 mg. and bluestone 500 mg. powder. The dietetic treatment adopted was as usual. In the enclosure where the sheep were kept a lick of common salt was accessible from the 23rd June, 1917, to the 30th June, 1917, and some other sheep, not dosed, were running in the same kraal. On the 29th June, 1917, a dosed sheep was found lying down, stiff in the hind quarters. There was a catarrh from the nostrils and the eyes were injected. A non-dosed sheep also showed stiffness in the hind quarters. As the sheep were in the open and the night had been cold it was supposed that the above ill-symptoms were produced by the low temperature.

Conclusions.—(1) In treating sheep that were left with free access to food and water one died out of twelve. (2) The administration of powder to such sheep did not result in the complete destruction of wire-worm infection. (3) The average dose of sodium arsenite, plus bluestone, given on two successive days, became toxic and dangerous when mixed with common salt. (4) The experiments on sheep that were allowed free access to a lick of common salt are not yet conclusive, but from the third conclusion it is evident that it would be a good precaution to remove such a lick when a flock is dosed. (5) In connection with the experiment showing the influence of salt on the dosed sheep, the following remarks made by Sir Arnold Theiler in a previous publication are of interest:—" . . . it is also possible that local conditions on the different farms may influence the toxicity of the mixture . . ." From the results recorded it can be surmised that one such condition could be the presence of "brakpans" (salt deposits).

F.—INFLUENCE OF THE WEATHER ON TREATMENT.

Summary of Experiments Nos. 57B, 57C, 67, 74, and 76.—In the experiments dealing with the dietetic regimen during treatment, it was shown that allowing sheep free access to food and water was dangerous for the sheep, and not completely effective on the wireworms, hence it was interesting to know whether rainy weather occurring during treatment would endanger the life of sheep or minimize the therapeutic result of treatment. A series of experiments on the above lines was difficult to arrange owing to the difficulty of forecasting the weather, but the necessary data were available from various other experiments that had been undertaken for different objects, but which are referred to in full in the following pages. During the progress of Experiment No. 57B, a heavy rain fell in the afternoon of the first and second days of treatment, with the result that pools of water were formed in the camp where the sheep were enclosed. The dose of sodium arsenite administered at that time, before the safe maximal dose was fixed, was a heavy one, but no ill effects were noted in the dosed sheep. In Experiment No. 57C, the afternoon rain was even heavier, and it was also very wet in the night after the second dose, but no ill effects were noticed in the treated sheep. In the experiment at Bestersput Farm (Orange Free State), when more than 1000 sheep were dosed, rain fell during the night for about four hours, but no cases of illness occurred. The effect of extreme temperatures was also taken into consideration, and it appeared that in very hot weather it was advisable to keep the sheep in the open or in a well ventilated stable. In regard to low temperatures, it appeared that there is difference in the resistance to the treatment between different species of sheep. In Experiment No. 76, young Merino lambs in the Orange Free State were dosed during a spell of cold weather, and were left in the open during the night. Some control lambs were also dosed, but were kept in a warm stable. Both lots of lambs stood the treatment equally well. In Experiment No. 74 some Persian sheep were dosed on a farm in the Cape Province (Norvals Pont) in the middle of the winter, and from the results it appeared that the cold weather had a harmful effect.

(a) RAINY WEATHER DURING TREATMENT.

Details of Experiments.

Experiment No. 57B.—On the 31st January, 1917, 232 adult sheep recently removed off a farm in the Transvaal, and in varying degrees of condition, were kept without food and water from 4 p.m. On the 1st February, 1917, at 9 a.m., they were dosed with sodium arsenite, 150 mg.; bluestone, 500 mg.; 100 of them being dosed with the powder and the remaining 132 with the liquid form. The sheep were kept in an enclosed camp on hard soil from which all vegetation had been removed. At 12 noon there was a very heavy storm, lasting for about one hour, and this was followed by a steady rain lasting nearly all the afternoon, with the result that in the enclosure numerous pools were formed with water 4 to 6 inches deep. The drinking trough was also half-full of clear rain water. At 4 p.m., while rain was still

falling, some green grass was given to the sheep, but they ate very little of it. In the evening the sheep were all wet through. On the morning of 2nd February, 1917, none of the sheep showed any ill effects. The second dose was given at 9 a.m., and at 1 p.m. the rain started again, lasting nearly all afternoon, pools again being formed. At 4 p.m., when it was still raining, hay was given to the sheep, but about 30 per cent. of them did not eat. They were kept under observation from 3rd to 6th of February, but no ill effects were noticed. *Remarks.*—It was observed in the above experiment that when the sheep got wet they did not drink water, and ate but little or no wet grass. It is, therefore, evident that the effect of the dosing was responsible for 30 per cent. of sheep not eating on the afternoon of the 2nd February, 1917. *Conclusions.*—Rainy weather does not interfere with the effect of the medicine on the sheep.

Experiment No. 57c.—On the 7th February, 1917, 124 sheep, in poor condition and anaemic, were dosed with sodium arsenite, 150 mg.; bluestone, 500 mg.; water, 10 c.c.; hydrochloric acid, 0.15 c.c. During the two afternoons of treatment there were heavy rainstorms, and heavy rain was also recorded in the night of the second day of treatment. For nearly all the time rain-water was lying in pools, and the drinking troughs were half-full. When grass was administered to the sheep during the two days of treatment, nearly 30 per cent. refused to eat. In the two afternoons when rain was collecting in pools and in the drinking troughs, the sheep were frequently kept under observation, but none of them were seen to drink water. No ill effects were observed in the treated sheep during the following days of observation. *Conclusions.*—The heavy rain in the two days of treatment did not produce any ill effects in the treated sheep.

Experiment No. 67.—This experiment will be fully reported in the paragraph dealing with "Treatment applied on the Farms," and it need only be mentioned here that on the 14th April, 1917, 473 sheep were dosed with the average dose in powder form, and 580 sheep were starved in preparation for the first dosing to be given next morning. At 7 p.m. on the same day rain fell, and continued for half the night. There was also a marked fall in the temperature. In the morning of the 15th April, 1917, the two lots of sheep were still wet, but the dosing was carried out as prearranged. No ill effects were noted in either lot of sheep.

(b) HOT WEATHER DURING TREATMENT.

No special experiments were undertaken to ascertain the effect of a high temperature during treatment, but this aspect is mentioned in some experiments recorded previously, and the results may be summarized here. The effects noticed were an increased thirst, some depression in the sheep, and rather frequent cases of diarrhoea. Many of the dosed sheep were kept in stables with a tin roof, where the temperature was as high as 40° C. In these cases the only observation worthy of note is that made in Experiment No. 50, where one death from heat-stroke occurred and two deaths from arsenical poisoning. It is questionable whether the dose would have been so fatal if the temperature had not been so exceptionally high, and the heat in the stable

so oppressive, and it appears, consequently, that during exceptionally hot days it is always better to keep the dosed sheep in the open than in a closed stable.

(c) COLD WEATHER DURING TREATMENT.

Experiment No. 76.—This experiment was carried out at Bestersput Farm (Orange Free State) in the month of July, 1917. The sheep used for the experiment were: (1) Ninety-five Merino lambs, about ten months old, thin and anaemic on account of heavy infestation with steek grass (*Aristida*), the smaller ones weighing from 45 to 55 lb. (2) Nine Merino lambs, about six months old, born on the farm. On the 5th July, 1917, at 5 p.m., the lambs came to the homestead from the pasture, and were put in an open kraal. In the night the minimum temperature was 9° C. The next morning the lambs were white with frost. On 6th July, 1917, at 9 a.m., the lambs were dosed, and at 11 a.m. they were sent out to pasture. The day was bright, with a maximum temperature in the shade of 15° C. At 5 p.m. the lambs came back from the pasture, when they were divided into two lots, one lot being put in a stable, while the others were left in an open kraal. During the night the minimum temperature in the open was 5° C., whilst in the stable it averaged between 15°-20° C. 7th July, 1917: Treatment was repeated the following day, when the maximum temperature was 18° C. In the evening the lambs were again divided in two lots as in the previous night, the minimum temperature recorded being 8° C. The following night the minimum temperature was again 8° C. All the lambs remained in good condition. *Conclusions.*—No ill effects occurred when the lambs were exposed to a minimum temperature of 9° C. during the time of treatment.

Experiment No. 74—With Persian sheep at Norvals Pont (Cape Province).—(a) On the 9th and 10th June, 1917, twenty Persian ewes, nearly all in lamb, and some heavy in lamb, were treated with the double dose. Ten ewes were rather poor in condition, and had an average weight of 55 lb.; the other ten ewes were in fairly good condition, with an average body weight of 81 lb. During the two nights of treatment the sheep were kept in a good pen. No ill effects resulted from treatment during the next few days. During the nights of the 11th and 12th the ewes were kept in an open kraal. The night of the 12th June was cloudy, windy, and cold; rain fell in the morning of the 13th June with a sharp drop in the temperature. At 7 a.m. of the same day a dry ewe was found markedly stiff in the hindquarters, and looking dull; the other dosed ewes were rather stiff and dull, but not so bad as the dry ewe. The day was sunny, and the temperature was mild. The dry ewe fed, and showed decided improvement. During the night of the 13th, this ewe was kept in a warm pen, and by the morning of the 14th had recovered. *Conclusions.*—The treated Persian ewes withstood treatment until they were exposed in the open during the cold night of the 12th. The stiffness that developed as a result of exposure improved under the conditions of a sunny day, and the warmth of the pen in the following night produced complete recovery in the one ewe.

Experiment No. 74.—(b) In this experiment forty-three Persian sheep and lambs were used, viz.:—Nineteen lambs, ten months old, weighing 32 to 42 lb.; one 2-tooth hamel; one 2-tooth ewe; twenty-two ewes in lamb, of which three were very heavy in lamb. The average weight of the ewes was from 52 to 74 lb. All forty-three sheep were dosed on the 12th and 13th of June with doses corresponding to their ages. In the afternoon of the 12th the sheep were normal and fed well. As mentioned in (a), the night of the 12th was cloudy, windy, and cold. Rain fell early in the morning of the 13th, with a sharp drop in the temperature. During the night of the 12th-13th June the sheep were in an open kraal. When the second dose was given the sheep looked miserable, were stiff and shivering, and it was thought that heavy mortality would be experienced. The other sheep on the farm that were not dosed, but were exposed to the same climatic conditions, were naturally wet, but otherwise normal. The day of the 13th was sunny and mild, and the sheep fed well in the afternoon, notwithstanding their stiff condition. As the night of the 13th promised to be cold, the sheep were put in a small, warm pen. On the 14th at 9 a.m., the sheep looked in excellent condition, and one ewe lambled.

Conclusions.—It would appear that it is not advisable to dose sheep during cold weather. Should an unexpected snap of cold weather be experienced in the middle of treatment the sheep should be kept in a warm pen during the inclement weather.

General Conclusions on the Effect of Cold Weather on Dosed Sheep.

Experiment No. 74 was carried out on account of a rather heavy loss that a farmer had experienced previously in dosing 400 Persian sheep on the same farm. The post-mortem examination made on some of the sheep that died showed pleuro-pneumonia in some cases and broncho-pneumonia in others. Some other ewes showed clinically either paresis or paralysis of the nervous motor and sensitive system. During the time of treatment the weather was very cold, but the farmer kept the sheep in the open. In comparing results of treatment carried out by the farmer and the observations made by the writer in the two above experiments, it would appear that the loss experienced by the farmer was due to the combined effects of treatment and exposure to cold weather. The observations made at Norvals Pont suggest that Persian sheep have a marked susceptibility to copper sulphate and sodium arsenite when the treatment is undertaken during cold weather, but more detailed observations are necessary before accepting this as a general experience.

NOTE.—The presumed susceptibility of Persian sheep may perhaps be explained by the physiological effect of arsenic. As arsenic has a retarding action on the general metabolism, particularly on oxidation, the result being a lowering of the body temperature, when sheep in this condition are then exposed to a very low temperature there is insufficient resistance against microbic or "afrigore" factors, with the result that pulmonary or nervous ailments develop. It can thus be understood that Merino sheep and Angora goats, with their thick coats, are better able to offer resistance to inclement weather conditions than the thinly coated Persian sheep.

General Conclusions on the Effect of Weather Conditions during Treatment.

(1) Rainy weather is well supported by Merino sheep when undergoing treatment. (2) Sub-tropical summer weather is not dangerous for sheep undergoing treatment, provided they are kept in the open. (3) Merino sheep withstand treatment well during the cold weather, provided they are not too poor in condition. (4) Persian sheep appear to show a special susceptibility to cold weather when under treatment. (5) In severe cold weather or in cold, rainy weather, it is advisable to give sheep protection in a pen during and a few days after treatment.

G.—COMPARATIVE EXPERIMENTS ON THE THERAPEUTIC VALUE OF SOME OF THE MORE COMMON DRUGS AGAINST WIRE-WORM INFECTION.

In South Africa bluestone was first advocated by the late Dr. Hutcheon. Lately Cooper's dip has been used on a wide scale, following the recommendations of Theiler, Gray, Cooper. Treatment with beta-naphthol was attempted by the present writer, and is referred to in this article. In America coal-tar creosote has been recommended by Stiles and Ransom. Bluestone is also frequently used on the lines followed in South Africa. However, gasoline is the more popular remedy. In Australia lysol is recommended by Reakes as the most efficacious remedy. In Europe lysol was advocated by M'Fadyean, creosote by Moussu, carbolic acid by Perroncito. Thymol was reported to have given good results in the hands of some practitioners in England. Arsenic was reported as the most effective by Craig. Picric acid was advocated by some authorities. In order to compare the results of the above drugs it was thought interesting to try their action on heavily infected sheep, taking care that the various sheep used were all of the same breed, in the same state of condition and infection, and were kept under similar conditions of environment.

Summary of Experiments Nos. 47 and 49.—In Experiment No. 47 lysol solution, gasoline and linseed oil, beech-tar creosote (instead of coal-tar, which was not procurable), were administered in the doses recommended by the various authors, and the results were compared with those given by sodium arsenite, plus bluestone, to the favour of the latter. In Experiment No. 49 lysol, beech-tar creosote, gasoline and linseed oil, thymol, beta-naphthol, were compared with sodium arsenite, plus bluestone, and the result again showed the advantage of the latter mixture.

Details of Experiments.—Experiment No 47 (9th October, 1916).—Eight Merino lambs of about 10 months of age, in fair condition, and just arrived from the Cape Province (Schoombie), were infected by an injection into the rumen of 10 c.c. watery suspension of H.C. larvae. On the 19th October, 1916, lamb No. 10496 was found lying down *in extremis* with typical acute haemonchosis, and was killed. On post-mortem examination the abomasum was coated with a thick layer of coagulated blood and mucus, in which H.C. in the fourth stage were very numerous. There were also rather

numerous adult H.C., evidently existing from an earlier natural infection. The other seven lambs also showed acute haemonchosis, cultures from the droppings showing good colonies of H.C. larvae, but these larvae were presumably from a previous natural infection. On the 21st and 22nd October the seven remaining lambs were treated with various drugs. In the treatment with creosote, two other lambs (4 to 5 months of age) were included and infected artificially. The results are given in the following table:—

No. of Lamb.	Weight in lb.	Drug Used.	Date when Killed.	Result.
10497	58	50 c.c. lysol 5 per cent. solution	4.11.16	Numerous H.C. in abomasum.
10493	50	" "	6.11.16	" "
10495	54	7 c.c. gasoline added to 15 c.c. linseed oil.	4.11.16	" "
10490	45	" "	4.11.16	No H.C. in abomasum.
10489	58	120 c.c. of a 1 per cent. beech-tar creosote solution.	4.11.16	Numerous H.C. in abomasum.
A	30	60 c.c. of a 1 per cent. beech-tar creosote solution.	1.11.16	" "
B	28	" "	22.10.16 (died)	" "
10494	43	Sodium arsenite 75 mg., bluestone 300 mg.	2.11.16	Not a single H.C. in abomasum.
10488	42	" "	6.11.16	" "

Remarks.—(1) The dose of lysol used for lambs Nos. 10497 and 10493 is that advised by M'Fadyean for adult sheep. (2) The dose of gasoline used for lambs Nos. 10490 and 10495 is that recommended by Ransom for lambs to be given for three consecutive days. In the present experiment gasoline was given for only two days, as it was considered that if the effects were not successful in this time it would have no advantage over the method now recommended with copper sulphate and sodium arsenite. (3) Coal-tar creosote was not used in the experiment as it was unobtainable in South Africa owing to war conditions. (4) The dose of beech-tar creosote used for lamb No. 10489 is that recommended by Stiles for lambs 4 to 12 months of age (maximal dose). The dose used for lambs A and B is the minimal dose. It must be noted that Stiles recommends a single dose, while in the present experiment the dose was used for two consecutive days.

Conclusions.—(1) Lysol, gasoline, and beech-tar creosote as used in the above experiment were not completely successful against wire-worms. (2) Gasoline had a positive effect in one case out of two. (3) The dose of sodium arsenite, plus bluestone, as recommended in the present work was completely successful against wire-worms.

Experiment No. 49.—On the 23rd October, 1916, a number of 10-month-old lambs that arrived from the Cape seven weeks previously (thin and rather pale) were examined, and cultures from droppings proved positive for H.C. infection. The same day they received an intra-ruminal injection of 5 c.c. watery suspension of H.C. larvae. On the 9th November, 1916, a lamb died with typical acute haemonchosis, showing on post-mortem examination in the abomasum a large number of H.C. that had just reached the fifth stage and a fair number of adult and old H.C. On the 11th and 12th of the same month the

lambs were treated with double doses of the various drugs, and the results are reported in the following table:—

No. of Lamb	Weight of Lamb.	Drug Used.	Date when Killed.	Result.
10467	40	50 c.c. lysol 5 per cent. solution	21.11.16	Very numerous H.C. in abomasum.
10472	43	" "	23.11.16	" "
10471	45	" "	20.11.16 (died)	" "
10485	43	100 c.c. lysol 5 per cent. solution	12.11.16 (died)	" " (died from lysol poisoning).
10466	42	" "	26.11.16 (died)	Very numerous H.C. in abomasum.
10458	40	120 c.c. of a 1 per cent. solution of beech-tar creosote	16.11.16 (died)	" "
10475	45	" "	23.11.16	" "
10469	40	" "	12.11.16 (died)	" "
10487	48	" "	21.11.16	" "
10483	46	" "	24.11.16	" "
10460	48	7 c.c. gasoline added to 15 c.c. linseed oil	23.11.16	Numerous H.C. in abomasum.
10479	50	" "	24.11.16	Some H.C. in abomasum.
10464	42	" "	21.11.16	8 males and 4 females of H.C. in abomasum
10474	45	" "	28.11.16	Numerous H.C. in abomasum.
10468	52	2 gm. powdered thymol	23.11.16	Fairly numerous H.C. in abomasum.
10462	48	" "	24.11.16	Numerous H.C. in abomasum.
10477	44	" "	21.11.16	Some H.C. in abomasum.
10484	45	2 gm. powdered beta-naphthol	21.11.16	Fairly numerous H.C. in abomasum.
10459	48	" "	23.11.16	Numerous H.C. in abomasum.
10480	45	" "	21.11.16	Very numerous H.C. in abomasum.
10463	42	75 mg. sodium arsenite and 300 mg. bluestone	21.11.16	Not a single H.C. in abomasum.
10476	44	" "	23.11.16	" "
10465	48	" "	24.11.16	" "
10470	45	" "	28.11.16	" "
10482	40	" "	28.11.16	" "

Remarks.—(1) In the abomasum of the lambs killed after treatment and found to be still infected with H.C., two kinds of wire-worm were found, viz.:—(a) Adult full-grown worms resulting from an old natural infection, and (b) worms that had just reached the fifth stage resulting from the artificial infection. It was pointed out in the paragraphs dealing with the "Action of the drug on the H.C. in Vitro," and it will again be referred to in a later paragraph (treatment by double initial dose followed by single monthly dose), that young worms are killed by a drug or by a dose of the same drug which is not able to kill the old worms. The fact that in the present experiment the drugs employed did not kill either the old or the young worms (with the exception of sodium arsenite, plus bluestone, which killed both) tends to show that the last mixture is by far the most effective of those used. (2) *Lysol*.—Lamb No. 10471 died eight days after treatment from acute haemonchosis. The abomasum showed an enormous number of young H.C. and large numbers of old H.C. There were coagula present to account for the lysol solution

failing to reach the worms. From the post-mortem examination it appeared that the treatment had no appreciable effect on the wire-worms. (3) Lambs Nos. 10485 and 10466 were dosed with 100 c.c. of a 5 per cent. lysol solution to see whether the dose could be increased. Lamb No. 10485, immediately after the first dose, started shivering and showed dyspnoea. Immediately after the second dose similar symptoms ensued, accompanied with slow pulsations, prostration, and death occurred within ten minutes. The post-mortem examination showed the typical lesions of haemonchosis with oedema of the lungs and foam in the bronchi. The mucosa of the abomasum was pale, thus showing that there was no cauterization from the lysol solution. A few H.C. were found dead, the remainder swimming. Death was due to acute lysol poisoning. Lamb No. 10466 died of acute haemonchosis fourteen days after treatment. (4) *Creosote*.—Lamb No. 10458. After the second dose it lay down, started to shiver, showed dyspnoea, but two hours later had recovered. Four days after, however, it died, apparently from acute haemonchosis. Lamb No. 10469.—Immediately after the first dose it commenced shivering, could not stand on its legs, and lay down for the rest of the day. The next morning it was found dead. The carcass was cold, and rigor mortis had set in when the post-mortem examination was made. Numerous H.C. were found in the abomasum, but were all dead. It appears that the lamb did not withstand the single dose of creosote. (5) *Gasoline*.—Lamb No. 10464 showed only old worms in the abomasum on post-mortem examination. The other lambs showed both young and old H.C. (6) *Thymol*.—Was given in powder form, followed by 50 c.c. of water. On post-mortem examination the lambs treated with thymol showed both young and old worms in the abomasum. (7) *Beta-Naphthol*.—Lamb No. 10480, which was very weak on account of strong haemonchosis, died three hours after the second dose. The other two lambs when killed showed young and old H.C. (8) *Sodium Arsenite, plus Bluestone*.—The five lambs that were dosed were all affected with an acute severe haemonchosis. No ill effects were noted during treatment, and no lesions which could be attributed to the drugs were found at the post-mortem examination. Lambs Nos. 10470 and 10482 showed a marked improvement before they were killed.

Conclusions.—(1) The administration of lysol in solution at the dose recommended in veterinary therapeutics was not effective against wire-worm infection in any of the three lambs treated. Lysol in a higher dose, and given for two days, was not only ineffective in both of the two lambs treated but it killed one of them. (2) Creosote appeared to be rather harmful in two lambs out of five, and one was killed apparently by a single dose. Judging from the number of worms found on post-mortem examination in all four sheep, it would indicate that the effect of creosote was very slight. (3) Judging from the number of worms found in the abomasum of lambs treated with gasoline, it would appear that its effect is superior to lysol, creosote, thymol, and beta-naphthol. It gave rather good results in two cases out of five. (4) Both thymol and beta-naphthol appeared to have an equally unsatisfactory effect. (5) Sodium arsenite, plus bluestone, gave complete results in destroying all the wire-worms in all the treated lambs, and the treatment was well supported even by lambs in the last stages of acute haemonchosis. (6) The drugs used in the present experiment could be arranged in a series and their action

expressed in percentages as follows:—Sodium arsenite, plus bluestone, 100 per cent., gasoline 70 per cent., beta-naphthol 55 per cent., thymol 50 per cent., creosote 20 per cent., lysol 10 per cent.

H.—COMPARATIVE ANTHELMINTIC VALUE OF SOME ARSENICAL COMPOUNDS.

In view of the marked anthelmintic superiority of sodium arsenite plus bluestone over the drugs discussed in the previous paragraph, an experiment was carried out to see if other compounds of the arsenical series possess the same vermifugal property as sodium arsenite. Three arsenical compounds mixed with bluestone, all in powder form, were tested, viz., arsenious oxide, arsenic oxide, and sodium arsenate, and compared with sodium arsenite. The doses used were the chemical equivalents for sodium arsenite.

Experiment No. 72.—On the 1st June, 1917, thirteen adult sheep were heavily infected, per rumen, with 20 c.c. of a watery suspension of H.C. larvae. Two infected sheep died of acute haemochosis on the 11th June, 1917, and on the 20th June, 1917, respectively. On the 3rd July, 1917, the remaining eleven sheep were weak, pale, poor in condition, and were showing severe haemonchosis when they were dosed for two consecutive days. The following table gives the results of treatment:—

No. of Sheep.	Arsenical Compound.	Date when Killed.	Result.
11351	Sodium arsenite 125 mg. Bluestone 500 mg.	26.7.16	Not a single H.C. in abomasum.
11376	" "	15.8.17	" "
K	" "	20.8.17	" "
11336	Arsenic oxide 135 mg. Bluestone 500 mg.	26.7.17	" "
10912	" "	31.7.17	" "
Z	" "	31.7.17	261 males and 80 females of H.C. found.
11418	Sodium arsenate 170 mg. Bluestone 500 mg.	21.8.17	18 males and 15 females of H.C. found.
11323	" "	30.7.17	19 males and 10 females of H.C. found.
Y	" "	1.8.17	Fairly numerous H.C. in abomasum.
10426	Arsenious oxide 100 mg. Bluestone 500 mg.	14.7.17	25 males and 12 females of H.C. found.
X	" "	30.7.17	Numerous H.C. in abomasum:

Conclusions.—From the above experiment it appears that none of the arsenical compounds used were as effective as sodium arsenite.

I.—METHOD OF DOSING SHEEP FOR WIRE-WORMS.

Having dealt with the chemo-therapeutic action of the drugs, and the dietetic treatment to be observed, the following remarks dealing with the technique of the operation are of importance from the practical point of view.

(a) POSITION OF THE SHEEP DURING DRENCHING.

In common with sheep farmers in other parts of the world, the South African farmer adopts one or the other more usual method of drenching, namely, either to hold the sheep in its normal standing position or to set it upon its haunches. The first method was advocated by Stiles in America as being that one in which "almost the entire quantity (of liquid) went directly into the fourth stomach," whereas "if the sheep was placed on its haunches, the fluid passed in part into the fourth stomach and in part into the first (the paunch)." The administration of the drench to an animal placed on its haunches has again been recommended recently in South Africa in a booklet on "Diseases of Sheep and Goats prevalent in South Africa," published by William Cooper & Nephews, "as the most satisfactory for dosing sheep with stomach or intestinal worms," because "the dose enters the fourth stomach more often than when dosed in any other position." In the present work the relative value of the two methods was studied, and a demonstration of the results is included in the table on page 396 with sheep Nos. 9711 to 9435. The first five sheep denoted by an \circ were dosed whilst on their haunches, and the other five denoted by \parallel were dosed whilst in a standing position. As can be seen, there was no practical difference in the result of the two lots of dosed sheep; the minimal and the maximal diffusion of the magenta, used to trace the passage of the drug, was nearly the same in both lots. Only in two out of ten sheep was the magenta mainly found in the abomasum, viz., in one sheep dosed whilst on its haunches, and one sheep dosed whilst in a standing position. In these experiments the liquid form was used because it is the only way in which it is swallowed at once when the sheep is still in the hands of the operator. In the two years in which this experimental work was conducted, the sheep were dosed in either the "standing" or "squatting" position, but no difference could be found in the effect of the drug. Towards the end of the work, however, the "standing" position was mainly adopted, as it was found that this position involved less strain on the natives holding up the sheep, a point which is worthy of consideration when a large number of sheep have to be dosed in the same morning.

Conclusions.—(1) Dosing sheep in a standing position or on their haunches does not have any appreciable difference in the passage of liquid drugs through the stomach. (2) The "standing" position is recommended on the grounds of facilitating labour.

(b) THE ADMINISTRATION OF THE DOSE IN POWDER FORM.

For the convenience of the sheep farmer, the finely ground powder, consisting of one part of sodium arsenite to four parts bluestone, is put up in tins containing sufficient for dosing 100 full-grown sheep.

He can also purchase spoons so constructed that when filled to the level of the edge with powder they hold the correct dose. Five sizes of spoons are supplied, differentiated by the number of "notches" on the handle, and holding the following quantities:—

No. of Notches on Spoon.	For Use on.	Quantity of Remedy.	Total.
One ...	Lamb, 2 to 4 months old ...	Sodium arsenite ... 36 mg. Bluestone ... 144 mg.	} 180 mg.
Two ...	Lamb, 4 to 6 months old ...	Sodium arsenite ... 50 mg. Bluestone ... 200 mg.	} 250 mg.
Three ...	Lamb, 6 to 10 months old ...	Sodium arsenite ... 75 mg. Bluestone ... 300 mg.	} 375 mg.
Four ...	Sheep, 2-tooth ...	Sodium arsenite ... 100 mg. Bluestone ... 400 mg.	} 500 mg.
Five ...	Sheep, 4-tooth and older ...	Sodium arsenite ... 125 mg. Bluestone ... 500 mg.	} 625 mg.

Sufficient powder, estimated roughly according to the number and age of the batch of animals to be dosed, is emptied into a round tin bowl, across the top of which a levelling blade is soldered. The correct spoon is selected, dipped into the powder in the bowl, withdrawn heaped up and then levelled by drawing it across the blade. The dose is then emptied into an ordinary teaspoon (to avoid getting the measuring spoon wet with saliva), and the teaspoon is emptied into the mouth of the sheep as far back on the tongue as can be conveniently reached. It is important to get the powder as far back on the tongue as possible, as if it is merely placed on the tip of the tongue the sheep are apt to spit it out. Even when the dose is given to a large flock by a careful operator, about 1 per cent. of sheep can be seen with greyish discoloured lips after dosing, indicating that part of the dose was wasted. In dosing sheep in the manner indicated above, the amount administered is fairly constant, but there is a margin of error, due not to variations in the size of the spoons, but to individual peculiarities in the way in which the measuring spoon is filled with powder. The writer has had many opportunities of observing this action in the hands of different operators, and the following table is illustrative:—

When Measuring with a Spoon of	Standard Dose should be	Results in the Hands of Different Farmers.		Maximum Dose when Spoon is "Tapped"
		Minimum Dose.	Maximum Dose.	
1 Notch	180 mg.	180 mg.	220 mg.	245 mg.
2 Notches	250 mg.	250 mg.	320 mg.	320 mg.
3 Notches	375 mg.	375 mg.	475 mg.	500 mg.
4 Notches	500 mg.	500 mg.	590 mg.	600 mg.
5 Notches	625 mg.	625 mg.	695 mg.	740 mg.

The dose indicated in "Maximum dose when spoon is tapped" was obtained by a farmer who, in error, tapped the spoon on the side of the bowl before levelling it off, thus getting the powder in a more compact mass than was intended. With the object of seeing if this action of "tapping" the spoon would interfere with the results of the dosing, the following experiment was undertaken:—

Experiment No. 57A.—On the 13th and 14th February, 1917, 125 adult sheep, rather poor and anaemic, were dosed with a mixture of sodium arsenite and bluestone in powder form in the proportion of 1 to 4. In measuring the dose the spoon was repeatedly tapped before passing it under the edge of the levelling blade. On the morning of the 15th one sheep was found dead. The hairs surrounding the lips were seen to be of a dirty greenish colour, showing that part of the dose was wasted. Post-mortem examination showed arsenical poisoning, but whether due to a high sensibility to arsenic or really to the high dose is doubtful. In the field the casualties resulting from "tapping" the spoon should, accordingly, not be too high, but, as it is unnecessary, it should not be adopted.

(c) COLOURING THE DOSE.

When a large number of sheep have to be dosed on farms where the equipment (i.e. kraals, labour, etc.) is not so complete as it is in the Laboratory, it was found that not infrequently dosed sheep were mixed with undosed sheep, with the result that some sheep were not dosed at all, whilst others received two doses in the same morning. The result in this latter case is death, and with a view to marking the dosed sheep in a distinctive manner some experiments were made to find out whether any dye could be added.

Experiment No. 68A.—To a mixture of sodium arsenite and bluestone (1 to 4) was added 1 per cent. of various aniline dyes, and twelve sheep were dosed with 625 mg. according to the following table:—

Aniline Dye Used.	Serial No. of Sheep Dosed.	Appearance of Mouth.	
		After Dosing.	An Hour after Dosing.
Fuchsin	1	Distinctly reddish	Clean.
"	2	" "	"
"	3	" "	"
Eosine	1	Slightly reddish	"
"	2	" "	"
"	3	" "	"
Imperial Blue	1	" "	Bluish tinge on the back of tongue.
" "	2	" "	Bluish tinge on lips.
" "	3	" "	Clean.
Cardinal Red	1	" "	"
	2	" "	"
	3	" "	"

Conclusions.—(1) Sheep dosed with the mixtures specified above could be distinguished from the non-dosed sheep shortly afterwards, but an hour later practically all traces of any colour had disappeared. (2) Imperial blue appeared to give the most satisfactory results.

Experiment No. 68B.—Sodium arsenite, plus bluestone in powder form (1 to 4), was mixed with various dye-stuffs, and after administration in the usual way the mouth was inspected from time to time till

all traces of the dye had disappeared. The result is given in the following table:—

Per Cent. of Dye Used.	Serial No. of Dosed Sheep.	Appearance of Mouth.				
		Immediately after Dosing (9 a.m.).	2 Hours later. (11 a.m.).	3 Hours later. (Midday.)	5 Hours later. (2 p.m.).	24 Hours later. (9 a.m.).
Violet ... 5 per cent.	1	Intense blue	Blue	Slight blue	Slight blue	No traces
	2	"	"	Blue	"	"
	3	"	Rather blue	Con. pap. blue	Con. pap. light blue	"
Violet ... 2 per cent.	1	Blue	Traces on con pap.	Traces	Traces	"
	2	"	Slight blue	Con. pap. blue	"	"
	3	"	"	Light blue	Con. pap. light blue	"
Gentian violet 5 per cent.	1	Violet	Traces	No traces	No traces	"
	2	"	No traces	"	"	"
	3	"	Violet	Violet	Violet	"
Orange ... 5 per cent.	1	Intense orange	No traces	No traces	No traces	"
	2	"	Orange lips	"	"	"
	3	"	No traces	"	"	"
Red ... 5 per cent.	1	Red	No traces	"	"	"
	2	"	"	"	"	"
	3	"	"	"	"	"
Black ... 5 per cent.	1	Rather black	Traces on con. pap.	"	"	"
	2	"	Few traces	"	"	"
	3	"	No traces	"	"	"
Brown ... 5 per cent.	1	Brown	No traces	"	"	"
	2	"	Traces on tongue	"	"	"
	3	"	Traces on lips	"	"	"

Remarks.—(1) When the sheep were examined at 11 a.m. (two hours after dosing) many of them were ruminating. (2) At 2 p.m. on the day of dosing the sheep were sent out to the pasture.

Conclusions.—From the above table it appears that the stain from violet 5 per cent., violet 2 per cent., and gentian violet 5 per cent. lasted longer than in any of the others.

Experiment No. 68c.—On the 27th April, 1917, the three dyes which gave the best result in Experiment No. 68B were tested again.

This time on a larger number of sheep. The result is given in the following table:—

Per Cent. of Dye.	Serial No. of Dosed Sheep.	Appearance of Mouth.				
		Immediately after Dosing (10 a.m.)	1 Hour later. (11 a.m.)	2 Hours later. (Midday.)	4 Hours later. (2 p.m.)	24 Hours later. (10 a.m.)
Violet ... 5 per cent.	1	Intense violet	Violet	Violet	Violet	No traces.
	2	"	Slight violet	Slight violet	Slight violet	"
	3	"	Intense violet	Intense violet	Violet	"
	4	"	Slight violet	Slight violet	Slight violet	"
	5	"	Intense violet	Intense violet	Violet	"
Violet ... 2 per cent.	1	Violet	Violet	Violet	Violet	"
	2	"	"	Slight violet	Slight violet	"
	3	"	"	Violet	"	"
	4	"	"	"	"	"
	5	"	"	"	"	"
Gentian violet 5 per cent.	1	Dull violet	Violet	Violet	Violet	"
	2	"	Intense violet	"	"	"
	3	"	"	"	"	"
	4	"	"	"	"	"
	5	"	Intense violet	"	"	"

Conclusions.—(1) At the time of dosing violet 5 per cent. and violet 2 per cent. were sharply marked, whereas gentian violet was not so distinct. (2) One hour after dosing violet 5 per cent. and gentian violet 5 per cent. were equally conspicuous, but violet 2 per cent. was still sufficiently conspicuous, and the stain remained in the mouth four hours after dosing. (3) The stain on the mouth had completely disappeared by the following morning, when the second dose was administered.

General Conclusions.—From the above experiments it appears that the addition of a 2 per cent. violet dye to the standard dose in

powder form enables farmers to distinguish dosed from undosed sheep for at least four hours afterwards, and in this way the danger of dosing sheep twice in the same morning is avoided.

(d) ADMINISTRATION OF THE DOSE IN LIQUID FORM.

It was thought that the more the liquid dose was reduced in volume the easier would be its administration, and it was found that the standard dose of either sodium arsenite or of bluestone dissolves completely in 10 c.c. of rain-water. If the two doses are mixed together in 10 c.c. of water the solution is turbid, but by the addition of 0.4 per cent. of hydrochloric acid, the solution becomes quite clear. It is thus quite possible to dissolve a packet of powder containing 100 single doses in a litre of water with the addition of about 14 c.c. of hydrochloric acid (1.13 to 1.16 specific gravity). In this liquid condition the medicine will remain fit for use for several months if kept in a well-corked glass or enamel vessel.

Position of the Sheep.—The observations made in this connection support the conclusion recorded in relation to the powder dose, in that the results are identical whether the liquid is given to a sheep standing in its normal position or when sat on its haunches.

Instrument for Dosing.—It was found that the liquid dose could be easily "squirted" into the mouth of the sheep by an ordinary graduated syringe of a 10 c.c. capacity. The liquid dose for the different ages of sheep is as follows:—For lambs 2 to 4 months, 3 c.c. of mixture; for lambs 4 to 6 months, 4 c.c. of mixture; for lambs 6 to 10 months, 6 c.c. of mixture; for 2-tooth sheep, 8 c.c. of mixture; for full-grown sheep, 10 c.c. of mixture. The dietetic method during treatment is similar to that adopted for the powder dose. In the opinion of the writer the effect of the liquid dose on the worms is perhaps slightly weaker than that of the powder dose, and the local effect on the mucosa of the stomach is also not so strong. The dose can be easily administered, and there is practically no danger of the dose passing into the lungs. In fact, some experimental dosings with liquid purposely undertaken in a very clumsy way with the object of "squirting" the solution into the trachea failed in every instance. However, to ascertain the result of the liquid passing into the lungs an intra-tracheal injection of the correct liquid dose was given to a 6-tooth Merino hamel in good condition. Previous to injection the respiration was 40, pulse 110, temperature 103. Immediately after injecting the sheep could hardly stand on its legs. The observations recorded subsequently were as follows:—*Five minutes later:* Foam and mucus from mouth, deep inspirations, short expirations, dilatation of nostrils. *Ten minutes later:* Groaning, pulse 140. *Fifteen minutes later:* Groaning at every expiration, temperature 103. *Thirty minutes later:* Sheep lying down, unable to stand, pulse 200, very weak. *Forty minutes later:* Sheep died, conjunctiva pale.

At the post-mortem examination the following lesions were noted:—Mucosa of trachea, hyperaemic; mucosa of bronchi, numerous petechiae; pleural cavity, 400 c.c. of clear yellow liquid; anterior and median lobe of both lungs, marked hyperaemia and oedema; large gelatinous clot and hydropericardium; epicardium, a few petechiae; left endocardium, some small ecchymoses; dark coagulated blood in right ventricle; kidneys, slight hyperaemia; conjunctiva and mucosa

of the mouth, both pale. From the above case it can safely be concluded that if the liquid dose passes to the lungs the sheep dies very quickly.

(c) EFFECT OF THE LIQUID DOSE ACCORDING TO THE MODE OF ADMINISTRATION.

Introduction.—In the paragraph dealing with the “passage of the drugs” in the alimentary canal of a sheep, it was seen that if a dose was administered through the mouth it passes to the anterior dorsal blind sac of the rumen and to the reticulum. Subsequently that portion of the drug in the reticulum passes to the abomasum, and the other portion in the anterior blind sac is mixed up with the rumenal contents, from where, gradually, and in a very diluted form, it passes to the abomasum. It was further seen that if the drug is injected into the rumen through the flank it is thoroughly mixed up with the rumenal contents and gradually passes to the abomasum. It can be easily seen that if the drug is injected directly into the abomasum it soon passes to the small intestine, with the exception of the portion absorbed by the mucosa. In administering the drug through the mouth the worms are first of all subjected to a rather concentrated solution of the medicine entering from the reticulum, and then to a second but weaker solution entering from the rumen. In injecting the drugs through the rumen the worms are only subjected to one action of a rather weaker solution. In injecting the drug through the abomasum the worms are subjected to a short but very strong action of the drug. It was thought interesting from the point of view of the administration of the dose to see which of the three above parasiticial actions was most effective on the H.C. located in the abomasum and less injurious to the sheep. In this connection the following experiment was carried out:—

Experiment No. 73.—On the 6th June, 1917, thirteen sheep were injected through the rumen with 20 c.c. of a watery suspension of H.C. larvae. From the 23rd June, 1917, to the 2nd July, 1917, two sheep died of severe haemonchosis, and the remaining eleven sheep all showed marked symptoms of haemonchosis. Nine of these sheep were then dosed on the 3rd and 4th July, 1917, while the other two (Nos. 9522 and Y) were dosed on the 25th July, 1917. The results are given in the following table:—

Dosed per Mouth.	Dose Injected into Rumen.	Dose Injected into Abomasum.	Date when Killed.	Result.
10954	—	—	27.7.17	Not a single H.C. in abomasum.
11118	—	—	4.7.17 (died)	H.C. all dead.
11196	—	—	21.7.17	Not a single H.C. in abomasum.
X	—	—	21.7.17	“ “
—	11039	—	8.7.17 (died)	Several H.C. in abomasum.
—	11126	—	20.7.17	Rather numerous H.C. in abomasum.
—	11017	—	28.7.17	Numerous H.C. in abomasum.
—	—	9459	4.7.17	Not a single H.C. in abomasum.
—	—	10612	4.7.17 (died)	“ “
—	—	9522	27.7.17 (died)	“ “
—	—	Y	26.7.17 (died)	“ “

Remarks.—(1) No. 11118 died in the afternoon of the second day of treatment. Post-mortem examination showed that death was caused by acute haemonchosis and no lesions of arsenical poisoning were present. The abomasum contained numerous H.C. all dead and whitish in colour, thus indicating that the wire-worms died previous to the death of the sheep. (2) Sheep No. 10612 was found dead in the morning of the 4th July, with an extensive haemorrhagic infiltration in the submucosa of the abomasum and a large necrotic area in the mucosa of the main curvature of the abomasum. Sheep No. 9459 was found sick in the same morning and was killed. Post-mortem examination showed universal hyperaemia of abomasum, numerous patches of haemorrhages in the submucosa and three small ulcerations. Sheep No. 9522 died on the day of treatment, and on post-mortem examination showed an intense gastritis with a large necrotic area and symptoms in other organs also indicating a plasma poisoning. Sheep Y, which died on the day after treatment, showed also haemorrhagic ulcerative gastritis, and a number of dead H.C. were found in the abomasum. The duodenum was normal; the jejunum and ileum showed marked enteritis; dead H.C. were found in the small colon. (3) In the present experiment 20 c.c. of a watery suspension of larvae was injected into the rumen of each sheep instead of 10 c.c. as was generally used in previous experiments. The infection which resulted was a very severe one, and presumably such as is never met with in the field. In fact, under the natural conditions on farms the larvae are picked up gradually in such a way that larvae and adult H.C. are not in a position to produce such a haemorrhagic and catarrhal gastritis, and thus give the parasites an opportunity of gaining protection against contact with the medicine. The two sheep that died of haemonchosis in this experiment showed a coating of mucus and coagulated blood on the abomasal mucosa.

Conclusions.—(1) The administration of the liquid dose per mouth was completely effective in all four cases. (2) The administration of the liquid dose through the rumen was ineffective in all three cases. (3) The administration of the liquid dose through the abomasum was completely effective on the worms, but killed three out of four treated sheep, whilst the remaining one showed on post-mortem examination after slaughter a very severe gastro-enteritis.

Notes and Considerations on the Technique of the Rumenal and Abomasal Administration of the Liquid Dose.

(1) *Rumenal Injection.*—In the course of the present experiments it was observed that when sheep are partially starved the rumen is somewhat collapsed, and it is dangerous to inject the dose into the peritoneal cavity. A sheep in which the dose was injected into the peritoneal cavity died within six hours of acute peritonitis and “plasma-arsenical poisoning.”

(2) *Abomasal Injection.*—From the numerous opportunities that occurred for studying the position of the abomasum in the abdominal cavity it was seen that unless this organ is full it is displaced by the pressure of the other portions of the stomach, so that if an injection is made slightly behind the xifoid appendix or a little below the right

costal arch the liquid would either enter into the reticulum or into the antero-dorsal blind sac of the rumen, or in between the two. Bearing this observation in mind, intra-abomasal injections referred to in this experiment were performed by laparotomy.

J.—TREATMENT APPLIED ON THE FARMS.

With the object of controlling the experimental results obtained at the Laboratory, and before introducing the method to the notice of the South African farmers, arrangements were made for the treatment to be carried out on a large scale on different farms in the Transvaal and in the Orange Free State.

(a) *Dosing undertaken on the farm Doornpoort, Pretoria District, situated about two miles from the Laboratory.*

The flock consisted of 396 sheep and 5 goats, mostly all in poor condition, some being even in extreme conditions of poverty. Six sheep, representative of the different grades of condition, were picked out and killed in order to obtain an idea of the degree of infection of the flock, the result of the post-mortem examination being summarized in the following table:—

No. of Sheep.	Condition of Sheep.	Number of H.C. found in Abomasum.	Presence of <i>Oes. col.</i> in Large Intestine.
9668	Poor	63 M., 90 F.	Fairly frequent.
9612	"	128 M., 164 F.	Numerous.
9691	Rather poor	28 M., 72 F.	Frequent.
8886	Fairly good. Anaemic	28 M., 39 F.	Nil.
9664	" " "	78 M., 66 F.	Fairly frequent.
8924	Good	Nil.	Numerous.

In connection with the above table, it is interesting to note that up to the date of the post-mortem examination on the above sheep on 10th October, 1916, no rain had fallen during the previous four months. On the 18th and 19th of October 390 sheep and 5 goats, all over 2-tooth, were dosed with sodium arsenite 100 mg. and blue-stone 500 mg. in powder form. One sheep in very poor condition was found dead in the morning of the 19th before the administration of the second dose, and on post-mortem examination showed marked anaemia and hydraemia. H.C. were fairly frequent in the abomasum. No symptoms of poisoning were noted. It was concluded that the sheep could not stand the treatment on account of marasmus. During the first day of treatment the weather was very hot and windy. No other ill effects resulted. On the 19th December, 1916, the flock on the farm consisted of 390 full-grown sheep, 4 goats, and 1 goat 10 months old. Good rains had fallen in November and December.

with the result that there was good grass on the farm and the sheep were in fairly good condition. At 10 a.m. on the same day the sheep were dosed with sodium arsenite 150 mg., bluestone 500 mg., water 10 c.c., hydrochloric acid 0.13 c.c. During the dosing of the flock a native assistant when holding open the mouth of sheep No. 9834 happened to draw the upper lip over its nostrils, and the greater quantity of the dose entered its lungs. The sheep started to cough and showed dyspnoea with foam dripping from the mouth and nostrils, and died at 1 p.m. the same day. Post-mortem examination showed acute arsenical poisoning. On the 20th December, 1916, the second liquid dose was given. No ill effects were noticed in the flock on that day and the day following. On the night of the 20th there was a thunderstorm with heavy rain. On the morning of the 21st two sheep in poor condition were found dead. On the night of the 22nd there was another heavy rainstorm, and on the morning of the 23rd two other sheep in poor condition were found dead. Post-mortem examination of the four sheep showed no lesions of arsenical poisoning, but of exposure and of anaemia and hydraemia. No worms were present in the abomasum. It was concluded that the dose did not cause death, but that the very poor condition of the sheep and the severe weather were responsible.

(b) *Treatment undertaken on the farm Maquabie, Amersfoort, south-eastern Transvaal, by Mr. A. G. Robertson on various dates in December, 1916.*

Experiment No. 45A.—The liquid dose was used, and the following extracts from various letters reporting on the progress of the experiment are sufficiently illustrative of the results:—

27th December, 1916.—“ We have dosed about 5000 sheep, including 2000 lambs. The lambs passed ‘milk-worm’ (*Stilesia centripunctata*) as I have never seen it done by any dose yet. From eight to sixteen hours after we gave them the first dose they passed hundreds.”

29th December, 1916.—“ We have dosed altogether 8000 to 9000 sheep of all ages. Out of all the sheep we dosed we have lost three after the first dose rather mysteriously, and I cannot account for it except that in handling big troops, unless great care is taken, they might after being dosed run back or get back somehow to the undosed ones and then get 20 c.c. which would kill them. I have examined several sheep that have died since being dosed, and in no case can we find wire-worms, whereas in every case of undosed sheep they can be found.”

Experiment No. 77.

(c) *Carried out at Potchefstroom under the supervision of Messrs. Mallinson and Michaelian, Sheep and Wool Experts of the Department.*

The experiment was divided into two parts:—(1) On the 6th, 7th, and 8th of January, 1917, an initial trial was carried out in dosing a small number of lambs and sheep. (2) The rest of the flock

was dosed on the 9th and 10th February, and the results of both experiments are given as reported by the supervising officers:—

“(1) On 8th January seventy-seven cross-bred, Merino, and Persian ewes, aged from 20 months to 5 years: twenty-one 6 to 8 month old cross-bred lambs; and twenty-four 4-month-old cross-bred lambs were yarded at 2 p.m., and got no water during the time of treatment. On the 9th, at 10.30 a.m., the above sheep were dosed with the prescribed doses of the bluestone and arsenite of soda mixture. Six hours after dosing these sheep were fed on the grass for one and a half hour and then returned to the kraal. On the 10th four samples of droppings were picked up in the yard indiscriminately. Under microscopic examination all these samples showed the presence of wire-worms. Tape-worms were also found in the yard amongst the droppings. One 4-month-old lamb that was sick before dosing died. Post-mortem disclosed advanced pneumonia; other organs and stomach normal. One Persian ewe 16 months old that was dosed with a full dose of bluestone and arsenite of soda died. Post-mortem disclosed pregnancy, intense inflammation of fourth stomach, the lining of which had peeled off. Lungs and heart also much affected. The above deaths took place between eighth and eighteenth hours after dosing. On the 10th, at 10.30 a.m., the second dose was given, the sheep being treated the same as the day before. On the 11th, at 10.30 a.m., these sheep were all well and were turned out to grass. General condition normal. Three more samples of droppings were examined under the microscope and showed dead wire-worms. No more tape-worms were found in the yard.”

Experiment No. 77 (2)—The results were tabulated by the officers concerned, and are given as reported.

Pen.	Class of Sheep.	Date Dosed.	Date Dosed.	Remarks, 7th.	Remarks, 8th.
Pen 1	4 cross-bred lambs, 4 months, 20 hours off food and water, fed on green feed for 2 hours daily, 8 hours after dosing, condition poor	6th Jan., 7 a.m.	7th Jan., 7 a.m.	At 7 a.m. on 7th January, tapeworms were found in excreta of 1 lamb. No visible wire-worms. No microscopic examination made	At 7 a.m. on 8th January, lambs look cut up in condition, but otherwise they are normal. Turned out to graze at 8 a.m. on 8th. No microscopic examination made.
Pen 2	4 poor cross-bred lambs, 7 months, fed as above	6th Jan., 7 a.m.	7th Jan., 7 a.m.	No visible tape or wire worms in excreta. No microscopic examination	Above remarks apply.
Pen 3	4 fair-conditioned cross-bred lambs, 7 months, fed as above	6th Jan., 7 a.m.	7th Jan., 7 a.m.	On 7th, at 7 a.m., 3 lambs had dropped tape worms. No microscopic examination	1 lamb scoured on 8th, at 7 a.m. Turned out to grass 8 a.m. on 8th.
Pen 4	4 Merino ewes, poor, over 2 years, fed as above	6th Jan., 7 a.m.	7th Jan., 7 a.m.	No visible worms at 7 a.m. on 7th in excreta. No microscopic examination made	3 ewes scouring at 7 a.m. on 8th; 2 dosed control killed showing dead wire-worms in small bowels.
Pen 5	4 Merino ewes, in fair condition, over 2 years, fed as above	6th Jan., 7 a.m.	7th Jan., 7 a.m.	No visible worms in excreta on 7th at 7 a.m. No microscopic examination made	1 control undosed had tape-worms and no wire-worms under the microscope. 3 ewes scouring on 8th at 7 a.m.
Pen 6	4 Merino ram lambs, 8 months, also 1 ram, 18 months, good condition. Starved for 20 hours before dosing and fed for 2 hours daily 8 hours after dosing	7th Jan., 7 a.m.	8th Jan., 7 a.m.	No visible worms in excreta at 7 a.m. Microscopic examination showed no wire-worms	8th, condition normal. Microscopic examination showed wire-worms dead in excreta.
Pen 7	4 Merino ewe lambs, 8 months, same condition as pen 6	7th Jan., 7 a.m.	8th Jan., 7 a.m.	Same remarks as pen 6 on 8th Jan. at 7 a.m. Microscopic examination showed dead wire-worms in excreta	8th, condition normal. Microscopic examination showed dead wire-worms in excreta.

Killed controls 8th January, 4 p.m.—2 ewes dosed both of which had dead wire-worms in excreta in small bowels under microscopic examination.

Killed undosed control.—1 ewe in good condition had no wire-worms under microscope, but was badly infected with tape-worms.

Sheep did not appear to be affected in any way by dosing, but looked a bit cut up after starving so long, which was mostly due to their low condition.

Scouring ceased on 9th instant, all sheep being normal.

Conclusions.—(1) The death of the Persian ewe after the first dose must be attributed to a quite exceptional susceptibility to arsenite or to the fact that the ewe was in an advanced state of pregnancy. (2) No ill effects were noticed in the dosed sheep with the exception of some cases of diarrhoea on the first day after dosing. (3) The vermicial effect of treatment is evidenced by the examination of faeces of sheep and by post-mortem examination on two treated ewes in which dead wire-worms were found in the small intestines. (4) The treatment proved to be also effective against tape-worms.

(d) *Undertaken on a farm in the Orange Free State in April, 1917, under the supervision of the writer.*

Experiment No. 67.—The flock consisted roughly of three grades:—(a) Sheep in good condition and probably free from wire-worm infection; (b) sheep in fair condition, the majority probably infected; and (c) 9-month-old lambs, very thin, anaemic, and all more or less heavily infected. The experiment was divided into two portions, consisting of 473 and 580 sheep respectively, and each batch was made up of representatives of different grades, in each grade being ewes, ewes in lamb, lamels.

A.—On the 14th April, 1917, dosing of the first batch of 473 sheep was undertaken, the dose being repeated the following day. It was observed that five sheep showed a greenish discoloration around the mouth, indicating that a part of the dose had been wasted. Rain fell on the evening of the 14th. One ewe from grade (a) died of pneumonia on 15th April, 1917, before administration of the second dose, and on post-mortem examination no H.C. could be found in the abomasum. No ill effects followed dosing.

B.—The second batch was again sub-divided into two lots:—(1) Consisting of 293 mixed sheep which were dosed on the 15th April, 1917, and 16th April, 1917, by the powder method, whilst the (2) remaining 287 were dosed on 15th and 16th April, 1917, by the liquid method. In the lot dosed with powder a few sheep showed greenish discolorations round the lips, but this fact was not observed in the lot dosed with liquid. No ill effects followed dosing.

Controls.—Three lamels and five lambs selected from the different groups were killed after dosing, but no H.C. were found on post-mortem examination.

Summary:—The following table, showing the number of sheep of various ages, etc., dosed, is perhaps of interest:—

Drugs Administered as	Number of Sheep and Lambs Treated.					Result.	
	8-Tooth.	2-Tooth.	6-10 Months.	4 Months.	2 Months.	Organotropic Action.	Parasitotropic Action.
Powder ...	663	6	85	9	3	No ill effects noted	In 6 sheep slaughtered.
Liquid ...	254		33	—	—	No ill effects noted	In 3 sheep slaughtered.
Total ...	917	6	118	9	3	Completely satisfactory.	Completely satisfactory.

NOTE.—The 663 8-tooth sheep dosed with the powder form consisted of:—

Hamels and Pregnant Ewes.	Ewes in Lamb.	Ewes Heavy in Lamb.	Ewes with Lamb 8 days old.	Ewes with Lamb 2-4 months old.
523	108	12	8	12

(e) *Treatment carried out at Bestersput Farm (Orange Free State) in Cold Winter Season.*

Experiment No. 76.—For reference to this experiment see paragraph "Influence of the Weather on Treatment."

(f) *Treatment carried out at Inhoek Farm (Cape Province) on Persian Sheep.*

Experiment No. 74.—This experiment was reported in full in the paragraph "Influence of the Weather on Treatment."

K.—ERADICATION OF H.C. INFECTION FROM A FLOCK AND FROM THE PASTURE.

Summary of Experiments.—A treatment which kills all the wire-worms in infected sheep affords the possibility of working out a method of treatment of a flock during the year which will eradicate the infection both from the flock and from the pasture. To work out this method it was important to know how long a treated sheep which runs on an infected farm can remain without being infected again, and what length of time elapses between the new infection and the presence of H.C. eggs in the droppings. From Experiments Nos. 60 and 61 it was found that practical results could be obtained by treating the sheep once a month. From Experiment No. 37 it appears that the double-dose treatment is perhaps too severe if repeated monthly, but from Experiment No. 71 it was concluded that a single dose repeated monthly is sufficient to kill all the worms acquired by sheep during the previous thirty days.

(a) *To see how soon sheep could be reinfected with wire-worm after administration of the double-dose treatment with sodium arsenite and bluestone.*

Experiment No. 60.—On the 21st February, 1917, and 22nd February, 1917, eight sheep were dosed each with sodium arsenite 150 mg. (in powder form) and bluestone 500 mg. (in powder form). Two sheep were infected 48 hours after the second dose, together with a control one which was not treated, and the remaining six sheep were infected at two days' intervals, with one control sheep in each

case. Some of the sheep were killed and the result is given in the following table:—

No. of Sheep Infected.	Interval between Treatment and Infection.	Result of Infection as shown by Post-mortem Examination.
11369	2 days	Not killed.
11396	2 days	Very numerous H.C.
11399	(Control)	" "
11421	4 days	" "
11440	4 days	Not killed.
11321	(Control)	Very numerous H.C.
11331	6 days	Not killed.
11335	6 days	"
11385	(Control)	"
11343	8 days	"
11408	8 days	Numerous H.C.
11330	(Control)	Very few H.C.

Conclusions.—From the above table it is evident that sheep can be reinfected 48 hours after treatment with the double dose.

(b) *To ascertain the interval that elapses between infection and the appearance of H.C. eggs in the faeces.*

Experiment No. 61.—On the 21st and 22nd of February, 1917, seven sheep and three lambs 3 to 4 months old were treated with the double dose, and on the 2nd March, 1917, the same sheep were infected with H.C. larvae through the rumen. The result of the observation is summarized in the following table:—

Interval between Infection and Examination.	Infection of Lambs.			Infection of Sheep.		
	Result of Examination of Cultures.		Result of Post mortem Examination.	Result of Examination of Cultures.		Result of Post-mortem Examination.
	Number Positive.	Number Examined.		Number Positive.	Number Examined.	
20	3	3	H.C. numerous, 30 per cent. laying eggs	Nil	5	—
21	1	1	—	Nil	5	—
22	1	1	—	Nil	5	—
33	1	1	—	1	5	—
24	1	1	—	1	5	—
25	1	1	—	2	5	—
26	1	1	—	4	5	—
27	1	1	—	5	5	—
31	1	1	—	(Not examined)		H.C. very numerous, majority ovipositing.
32	1	1	H.C. very numerous, majority ovipositing	—		—

Remarks on the above table.—(1) Twenty days after infection a lamb died of haemonchosis. The abomasum was swarming with H.C. in adult stage, of which 30 per cent. were in oviposition. In the faeces, eggs of H.C. were fairly numerous. Some droppings were placed in culture and fairly numerous larvae were found on the walls four days later. On the same day (20th) cultures of droppings were made from the other two lambs, and larvae of H.C. were also found four days later. (2) Twenty-one days after infection one lamb had severe diarrhoea and it was not possible to make any further cultures from it. (3) In the sheep a few larvae were found in cultures from the 20th day onwards, but until the 23rd day it was not possible to say if these were larvae of H.C. or of *Oes. col.* or some other nematodes. The aspect of the culture on the 23rd day, however, justified the conclusion that the colonies then found resulted from artificial infection. (4) In the night of the 31st day a sheep died of acute haemonchosis as a result of the heavy infection.

Conclusions.—(1) Young lambs passed H.C. eggs in the faeces twenty days after infection with H.C. larvae. (2) In adult sheep it is difficult to say whether the first few eggs passed in the faeces, after a reasonable time has elapsed since artificial infection, are those of the injected worm or are from some other infection. On the 23rd day after infection the presence of H.C. eggs in the faeces was detected with certainty in 20 per cent. of the cases, and on the 31st day numerous eggs were present in the faeces of all the sheep examined. (3) Eggs of H.C. are fertile as soon as the female starts ovipositing. (4) From the above experiment it appears that to eradicate wire-worm infection in adult sheep not more than 20-30 days should elapse between successive treatments. (5) From the result of infection in young lambs it would appear that they should be dosed every 17-20 days, but as under ordinary farming conditions such young lambs are very seldom heavily infected there is no need to apply this principle in practice. (6) It must be remembered that the parasitic evolution of H.C. in adult sheep in 23 days probably represents the shortest period.

(c) *To ascertain if sheep can stand a double-dose treatment administered approximately once a month.*

Experiment No. 37.—114 lambs 9 to 10 months old were selected in June, 1916, at a time when the wire-worm infection was practically absent, and equally divided into two batches, one batch being reserved for periodical dosing and the other lot kept for control purposes..

The details of the experiment and the result are given in the following table:—

Day of Treatment.	Number of Lambs Dosed.	Number of Lambs not Dosed.	Dose Administered.	Average Weight in lb.		No. of Deaths.		Cause of Death.
				Dosed.	Undosed.	Dosed.	Undosed.	
24.6.16	57	57	Cooper's dip 500 mg., bluestone 500 mg.	43	46	3 (between 11th and 17th July)	3	Poverty and exposure.
11.8.16	51	54	Sodium ars. 75 mg., bluestone 300 mg.	45	48	—	—	—
18.9.16	54	57	Sodium ars. 75 mg., bluestone 400 mg.	47	50	—	—	—
20.10.16	50	50	Sodium ars. 75 mg., bluestone 300 mg.	48	52	—	—	—
5.12.16	50	50	Sodium ars. 90 mg., bluestone 300 mg.	49	54	—	1 (8.12.16)	Interstitial pneumonia.
Lambs clipped on 16.12.16	48	48	—	46	51	—	—	—

Remarks on the above table.—(1) During the month of September, 1916, three new lambs were added to the lot, so that the number of untreated lambs on the 18th September, 1916, again appears as 57. (2) Of the 50 lambs dosed on the 5th December, 1916, twenty had all the milk teeth, and thirty were showing the two median teeth in various stages of growth. (3) On the 16th December, 1916, the dosed and undosed lambs were clipped when the opportunity was taken of weighing them. The dosed lambs were obviously thinner than the undosed lambs.

Conclusions.—A monthly treatment with a double dose does not cause any mortality, but retards the normal growth of the sheep.

(d) *Experiments undertaken with the object of determining whether a flock of sheep can be kept clean by adopting an initial double-dose treatment and subsequent single-dose treatment once a month.*

Experiment No. 64.—Sheep were dosed at various intervals between 25 and 60 days after infection, and killed later, when the effect of the treatment on the wire-worm infection could be determined.

On the 1st and 2nd April, 1917, seven sheep in fair condition were treated with a dose of sodium arsenite, 125 mg., in powder form, and bluestone, 500 mg., in powder form, to kill off all the old worms, and to leave the sheep clean so that the subsequent artificial infection could be traced. On the 5th April, 1917, they were injected through the rumen with 10 c.c. of a watery suspension of larvae, and were afterwards allowed to graze on the veld, receiving every morning and evening a feed of green lucerne. Two control sheep were killed on the 29th and 44th days, respectively, to ascertain the presence of infection of the flock, whilst the remaining five sheep were dosed at different intervals. The result of the experiment is summarized in the following table:—

No. of Dosed Sheep.	No. of Sheep not Dosed.	Number of Days between Infection and Treatment.	Number of Days between Infection and Death.	Result at Post-mortem Examination.
11340	—	25	29	Not a single H.C. Abomasum pale.
—	11331	—	29	200 H.C. <i>Perechiae</i> in abomasum.
11371	—	40	44	Not a single H.C.
—	11362	—	44	Numerous H.C. Large patches <i>Tric. ext.</i>
11303	—	48	51	Not a single H.C. Small patches of <i>Tric. ext.</i>
11410	—	54	56	Some H.C. All males.
11352	—	60	65	Not a single H.C. 30 <i>Oes. col.</i> in colon.

Remarks.—(1) Sheep No. 11303 was the thinnest and most anaemic of the lot. Eggs were found to be frequent in the faeces. (2) Sheep No. 11352 on the day of treatment, 60 days after infection, was pale, anaemic, in poor condition, and showed typical haemonchosis. Cultures made from the droppings showed a very rich colony of larvae. (3) In the column headed "Result at post-mortem examination," is mentioned the presence of patches of *Tric. ext.*, meaning that in the pyloric area of the abomasum were found patchy and roughened catarrhal areas on the mucosa where the *Tric. ext.* were frequent.

Conclusions.—The administration of a single dose of the remedy in powder form 48 days after artificial infection was effective—after an interval of 54 days the dose was not completely effective, but the presence of males of H.C. only means that the dose was nevertheless destructive on the females of H.C. It is interesting to note the complete effect on the 60th day after treatment in a case which was heavily infected.

Experiment No. 71 was undertaken with a similar object to Experiment No. 64, i.e. to note the effect of a single dose given at intervals of 22 to 26 days. On the 31st May, 1917 and 1st June, 1917, ten sheep in rather poor condition were dosed with sodium arsenite, 150 mg.; bluestone, 500 mg. On the 4th June, 1917, the sheep were injected in the rumen with 20 c.c. of a watery suspension of H.C. larvae. Sixteen days later the sheep were showing very acute symptoms of haemonchosis, and were treated with a single dose at

varying intervals. The results of the treatment and of the condition of control sheep are given in the following table:—

No. of Dosed Sheep.	No. of Un-dosed Sheep.	Number of Days between In-fec-tion and Treatment.	Number of Days be-tween In-fec-tion and Death	Dose Given.	Result at Post-mortem Examination.
11469		22	25	Sodium arsenite 125 mg. Bluestone 500 mg.	Not a single H.C. in abo-mastm.
11442		22	29	" "	37 males and 10 females H.C.
—	X	—	23	Control	Very numerous H.C. and coagula.
11436	—	26	43	Sodium arsenite 125 mg. Bluestone 500 mg.	Not a single H.C. in abo-masum.
9451		26	44	" "	Some H.C.: numerous coagula.
9513		26	47	Sodium arsenite 150 mg. Bluestone 500 mg.	Not a single H.C. in abo-masum
11360	—	26	47	" "	Some H.C.: numerous coagula.
—	9446		47	Control	Very numerous H.C. in abomasum.

Remarks.—(1) In experiment No. 64 the sheep were injected with 10 c.c. of larvae in water, and the injection resulted in a severe infection. In Experiment No. 71 the sheep were injected with 20 c.c. of larvae, as it was surmised that owing to the cold weather the number of larvae present in a 10 c.c. of a watery suspension would be insufficient to ensure an adequate infection. The result was, however, that the infection was exceptionally severe, seeing that on the 23rd day, when sheep X was killed, the mucosa of the abomasum was still coated with a layer of blood coagula.

(2) The result of the administration of a dose twenty-six days after infection supports the above statement, as sheep 11436 and 9513, which had no thick coagula in the abomasum, where H.C. could find shelter, were completely freed from wire-worm, whereas sheep 9451 and 11360, which, even after dosing, showed numerous coagula, were not completely clean.

Conclusions.—(1) The conditions of infection of the sheep in the above experiment were more severe than is met with under ordinary farming conditions in naturally infected sheep. (2) In such exceptional cases the single dose would not be completely destructive on the H.C. sheltered in the coagula.

TREATMENT OF GOATS FOR WIRE-WORMS.

Experiment No. 59A.—Five goats in fair condition, and from 2 to 8 tooth, arrived from Doornpoort early in the month of January, 1917, and since their arrival had been kept in the stables of the Laboratory.

On the 5th and 6th January, 1917, the goats were dosed with sodium arsenite, 125 mg., in powder; bluestone, 500 mg., in powder. Dietetic regimen during treatment was as usually recommended. One goat took the powder very badly and coughed a lot. Up to the 16th January, 1917, no ill effects were noted in the goats, and they were discharged.

Experiment No. 59B.—On the 10th and 11th January, six goats, from 4 to 8 tooth were dosed with sodium arsenite, 150 mg., in powder; bluestone, 500 mg., in powder. Some of the goats were very restless during dosing. Up to the 16th January no ill effects were noted in the treated goats.

Experiment No. 59c.—On the 19th-20th January one goat (7-tooth), in good condition, was dosed with sodium arsenite, 150 mg., in powder; bluestone, 500 mg., in powder, and on the same days three others, 6-8 months old, of which one (10939) was in very poor condition, and suffering from intense diarrhoea, were dosed with sodium arsenite, 75 mg., bluestone, 250 mg., in powder. No. 10939 died on the 21st January, and post-mortem examination showed marked anaemia and hydraemia; no local lesions of arsenical poisoning were detected. The other three dosed goats did not show any ill effects.

Experiment No. 59D.—On the 16th and 17th January, 1917, six goats, 8-tooth, were dosed with sodium arsenite, 150 mg.; bluestone, 500 mg.; hydrochloric acid, 0.45 c.c.; water, 10 c.c. One goat was poor, dull, with diarrhoea before treatment, and showed the same after treatment. The other goats did not show any ill effects.

Experiment No. 59E.—On the 19th and 20th December, 1916, four adult goats were dosed with same dose as stated in Experiment No. 59D. In addition one goat, 10 months old, was dosed with 6 c.c. of the same solution. Treatment was carried out at Doornpoort farm. No ill effects were noticed in the treated goats.

Experiment No. 59F.—On the 4th and 5th of May, 1917, twenty-four goats from 6 to 8 tooth were dosed with sodium arsenite, 125 mg., in powder; bluestone, 500 mg., in powder; three kids from 6 to 10 months old were dosed with sodium arsenite, 75 mg., in powder; bluestone, 300 mg., in powder; and one kid, between 4 and 6 months old, was dosed with sodium arsenite, 50 mg., in powder; bluestone, 200 mg., in powder. The goats were brought from Doornpoort and dosed in a stable of the Laboratory. After the first dose half of the goats showed loss of appetite. On the 7th May, 1917, all the goats were all right.

Conclusions.—Goats, being more excitable than sheep, are better dosed in the standing position. The dose issued by the Laboratory, either in powder or in liquid form, was well supported by all the goats dosed.

The Fate of Ingested and Injected Arsenic in Sheep, with Special Reference to Treatment of Haemonchosis.

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From the Onderstepoort Laboratories for Veterinary Research.

Pretoria, January, 1918.

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I.—INTRODUCTION.

THE value of arsenical preparations for destroying wire-worm (*Haemonchus contortus*) in infected sheep has been recognized now for many years, but owing to their poisonous character and uncertainty as to the best conditions under which they might be effectively and safely administered, arsenical remedies have not always met with that success and popularity justified by their potential virtues.

Their usefulness was well recognized by Theiler, who frequently recommended them in conjunction with copper sulphate, a compound earlier advised by Hutcheon as a vermifuge. Cooper's dip, a preparation containing soluble arsenite and sulpharsenite, had a considerable vogue in South Africa, and in 1912 Theiler* published the results of experiments designed to determine the safe dose of Cooper's dip and of "white arsenic," when mixed with copper sulphate. As a result of these investigations and of practical experience in the field, the use of 10 grains each of Cooper's sheep dip and copper sulphate was recommended† as a medium dose for adult sheep, 7 grains each for two-tooth sheep, and 5 grains each for lambs of six to nine months. The maximal safe dose was regarded as being about 50 per cent. higher. The dose recommended for adult sheep thus corresponds to about 0.65 grm. of copper sulphate, and soluble arsenite equivalent to 0.13 grm. As_2O_3 .

The arsenic was recommended in the form of Cooper's dip for the reason that this material was already well-known and easily procurable in all parts of the Union. In making his recommendations Theiler stated that the treatment did not completely eradicate all the wire-worms, but was useful as a means of reducing their number.

At this stage the work was taken up by Veglia,‡ who in 1915 published a detailed study of the anatomy and life-history of the worm.

* "Experiments to Determine the Safe Dose of White Arsenic, Cooper's Dip, and Bluestone for Sheep." *S. African Agric. Journ.*, March, 1912.

† Interim Report *re* Dosing of Sheep with Cooper's Dip and Bluestone. No. 48, 1912, Dept. of Agric., Union of South Africa.

‡ "The Life-History and Anatomy of *Haemonchus contortus*." Third and Fourth Reports of the Director of Veterinary Research, 1915.

Following out the information gained concerning the life-history, further experiments were initiated on drug treatment, and an improved method of dealing with wire-worm infection was worked out. The details of Veglia's experiments are given in his own paper in this present (Fifth) *Report of the Director of Veterinary Research*, but the net result may be summarized as a vindication of soluble arsenite and copper sulphate in the proportions advocated by Theiler, but administered upon a plan, based upon the life-history of the worm, which enables a practically complete clearance to be effected. This system of dosing, after trial on the large scale in the field, has been made by Theiler the basis of the official recommendations for the control of wire-worm in the Union.

The present paper deals with the work carried out in the chemical laboratory, as a collateral branch of the general dosing experiments, in order to gain information concerning the fate of arsenic after ingestion by the sheep. Some of the sheep concerned were those utilized by Veglia and passed on to us from the post-mortem table, while others were put into experiment with the express object of following the path of the dose and the rate of elimination of the arsenic. The general scientific interest of securing such data is obvious, but the practical significance of the work will be clearer to the chemist reader if the wire-worm treatment, now recommended by the Division of Veterinary Research, be understood. The leaflets dealing with the matter, and issued to farmers by the Department of Agriculture, are therefore attached in appendix form to this communication. Appendix A gives the instructions provisionally stereotyped early in 1917 after field trial, for use with the "wire-worm remedy" now supplied to farmers by the Division. Appendix B is a supplementary memorandum issued later in the year as a result of extended experience, recommending slight alteration and simplification of the original treatment. Appendix C is a short popular presentation of the life-history of the worm, abstracted from Veglia's 1915 paper.

The "wire-worm remedy" itself, to which the instructions of Appendix A apply, consists of an intimate mixture of copper sulphate and arsenite of soda (the usual high-grade commercial article, 80 per cent. As_2O_3) in the proportions of 4 : 1, of standard fineness (passing a sieve with 60 meshes to the linear inch), put up in tins each containing sufficient powder (62.5 grm.) for the single dosing of 100 adult sheep. Along with this, standard hemispherical measuring spoons are sold in sets of five made to deliver, as nearly as possible, .625, .500, .375, .250, .200 grm. of powder respectively. These quantities correspond to 100, 80, 60, 40, 32 mg. of arsenious oxide in the form of soluble arsenite, and are adapted to animals ranging from adult sheep to two-month-old lambs in accordance with the instructions of Appendix A. Each batch of remedy is tested before issue to control arsenic content and concordance with spoon volume, and so ensure that possible errors in dosing are maintained within permissible limits. Some idea of the popularity which the remedy has achieved amongst the farmers is given by the fact that in this first year of issue well over six million doses have been made up and dispatched from the Laboratory. Excellent results are reported throughout the Union, and the casualties have been altogether negligible.

II.—ESTIMATION OF ARSENIC.

For the purpose of the present work it was necessary to estimate small amounts of arsenic as accurately as possible, in order that the distribution and elimination of the known small quantities administered might be followed with a reasonable degree of certainty. A micro-titration method of our own devising was finally adopted in preference to either the Marsh mirror or the Gutzeit colorimetric test, but since the plea for this procedure is advanced in a separate paper* it need not be elaborated here. Correction curves were utilized for bringing the data as near the truth as possible, and a degree of accuracy of ± 5 per 100 is claimed for the results. Where quantities below one-twentieth of a milligram were present in a workable quantity of material taken for analysis, the error is higher, and results are then either put down as "trace" or in proximate figures (sometimes checked by colorimetric determination) according to the significance attaching to them. A "trace" may be read as anything below a few parts per ten million. Such quantities are of little significance to the investigation.

III.—DISTRIBUTION OF ARSENIC IN THE ALIMENTARY SYSTEM.

The data showing the distribution of arsenic in the alimentary system of the sheep under different conditions of administration are summarized in Table I. The treatment of the sheep is indicated in the second vertical column and need not be detailed seriatim. It may be added that all the sheep were in good condition, and all fully grown, with the exception of No. 10576, which was of two-tooth age, and No. 8969, which was four-tooth. With the exception of No. 11387, which was injected through the flank with acidulated aqueous solution, the dosing was carried out with the material in powder form, and with the sheep held back on their haunches as they would be in dosing practice. In Table I the analytical data are rounded off in whole milligrams As_2O_3 , wherever permissible.

TABLE I:

ENSUING PAGES 488 AND 489.

* Green: "The Micro-titration of Arsenic." This volume (fifth) of the Report of the Director of Veterinary Research.

Table I.

Showing the distribution of arsenic over the alimentary tract in sheep dosed with safe quantities of wire-worm remedy, and killed for investigation at various intervals after dosing.

Sheep No.	Treatment of the Sheep.	Rumen.		Reticulum.		Omasum.		Abomasum.		Total in Stomach System expressed as per cent. of Administered	First Intestine.		Second Intestine.		Liver.				
		Weight of Contents.	Arsenic Found As_2O_3 .	Weight of Contents.	Arsenic Found As_2O_3 .	Weight of Contents.	Arsenic Found As_2O_3 .	Weight of Contents.	Arsenic Found As_2O_3 .		Weight of Contents.	Arsenic Found As_2O_3 .	Weight.	Arsenic Found As_2O_3 .					
		grm.	mg.	grm.	mg.	grm.	mg.	grm.	mg.		grm.	mg.	grm.	mg.	grm.	mg.			
8325	Starved 16-18 hours, dosed with bluestone and Cooper's dip; killed 2 hours later.....	2,350	15	65	2.0	55	14.0	260	54	81	Nearly empty	Trace	520	2.0	670	Trace (0.3)			
8133	As with 8325, but killed 4 hours after dosing.....	2,000	6	150	4.5	45	4.5	150	26	39	190	3.0	570	2.0	325	1.5			
8969	As with 8325, but allowed green food without water 5 hours after dosing; killed 24 hours after dosing.....																		
10676	Starved 16-18 hours, dosed with bluestone and commercial arsenite of soda; killed 5 hours after dosing.....	2,800	13	90	7.0	70	2.0	300	7	28	680	3.5	600	12.0	690	1.7			
10638	As with 10676, but killed 12 hours after dosing.....	2,740	14	120	4.5	—	15.0	820	36	58	350	1.5	675	Trace	450	1.4			
9725	As with 10676, but offered green food 5 hours later; very little eaten; killed 24 hours after dosing.....	3,320	15	118	3.0	50	4.5	200	4	22	400	1.5	480	1.5	425	1.1			
10641*	As with 10676, but given green food 5 hours after dosing; starved again until the following morning; then fed and watered as usual; killed 48 hours after dosing.....	1,550	26	100	2.0	Omasum and Abomasum.										400	Trace	405	0.5
						Total arsenic = 5 mg. As_2O_3										—	Trace	490	0.7
10753	As with 10676, but fed 5 hours after, then starved another 16-18 hours; dose then repeated and green food again offered 5 hours later; killed 12 hours after second dose..	1,500	1	95	Trace	Total arsenic = Trace										500	1.5	490	1.2
		2,750	27	130	1.3	60	4.0	150	Trace	(13)	390	1.5	550	14.0	490				
		120 (twice)																	

* Part of the arsenic passed in the urine before death was determined. The data are discussed in the text.

Table I—(continued).

Showing the distribution of arsenic over the alimentary tract in sheep dosed with safe quantities of wire-worm remedy, and killed for investigation at various intervals after dosing—(continued).

Sheep No.	Treatment of the Sheep.	Arsenic Equivalent of the Dose As_2O_3 .	Rumen.		Reticulum.		Omasum.		Abomasum.		Total in Stomach System expressed as per cent. of the Dose Administered.	First Intestine.		Second Intestine.		Liver.	
			Weight of Contents.	Arsenic Found As_2O_3 .	Weight of Contents.	Arsenic Found As_2O_3 .	Weight of Contents.	Arsenic Found As_2O_3 .	Weight of Contents.	Arsenic Found As_2O_3 .		Weight of Contents.	Arsenic Found As_2O_3 .	Weight of Contents.	Arsenic Found As_2O_3 .	Weight.	Arsenic Found As_2O_3 .
		mg.	grm.	mg.	grm.	mg.	grm.	mg.	grm.	mg.		grm.	mg.	grm.	mg.	grm.	mg.
10763*	As with 10753, but fed and watered as usual 24 hours after the second dose; killed 3 days after second dose....	120 (twice)	3,460	2	200	Trace	95	Trace	275	Trace	(1)	330	Trace	530	4.0	550	Trace (0.2)
10576	Starved 16-18 hours, dosed with bluestone and arsenite of soda; offered green food 5 hours later; killed 8 hours after dosing.....	100	2,540	43	55	0.6	18	1.0	135	3	48	250	1.0	550	Trace	480	1.5
10680	Duplicate of 10576.....	100	1,480	7	91	1.0	38	2.5	255	30	41	250	1.0	775	Trace	395	3.5
10868*	Starved 16-18 hours, dosed as in case of 10576, but given no food thereafter; also killed 8 hours after dosing.....	100	1,870	15	53	0.7	24	1.6	525	16	33	—	—	—	—	485	3.6
11362	Not starved, but dosed with bluestone and arsenite without preliminary treatment; then allowed green food <i>ad lib.</i> , but no water; killed 8 hours after dosing.....	100	5,020	62	20	Trace	31	0.5	340	2	65	—	—	—	—	440	2.3
9705	Starved 16-18 hours, dosed with bluestone and arsenite; given no food, but drenched with 200 c.c. water 3 hours later, and another 100 c.c. after another 3 hours; killed 8 hours after dosing.....	100	4,600	62	98	1.8	87	2.7	30	Trace	67	—	—	—	—	580	0.8
11387	Bluestone and arsenite of soda dissolved in water acidulated with HCl; 10 c.c. injected direct into the rumen; allowed food 5 hours later; killed 8 hours after injection.....	100	4,220	69	387	7.0	68	1.0	375	3	80	—	—	—	—	690	Trace (0.3)

* Part of the arsenic passed in the urine before death was determined. The data are discussed in the text.

Path of the Dose.—Perhaps the most noticeable feature of these data of Table I is the irregular fashion in which the dose may be distributed over the stomach system. With Nos. 11362 and 9705, 62 per cent. of the arsenic in the dose was still present in the rumen eight hours after administration. This figure, taken in conjunction with the 69 per cent. recovery for No. 11387, where the dose was injected quantitatively into the rumen, indicates that in the two cases concerned practically the whole of the dose swallowed was first passed into the rumen. No. 8325, on the other hand, represents a case in which the greater part of the dose evidently passed into the reticulum and thence into the abomasum. Two hours after dosing 81 per cent. of the total arsenic administered was still recoverable in the stomach system, but of this less than one-fifth was found in the rumen. With No. 8133 still less would seem to have passed into the rumen since only 6 mg. is recovered there four hours after administration. The path in this case would seem to have been through the reticulum into the abomasum and thence fairly rapidly on into the intestines. The abomasum still contains a quarter of the dose given, but over 60 per cent. has passed out of the stomach system altogether.

Little constancy is shown, and the channel taken by the dose seems arbitrary. This is interesting in connection with Veglia's observation that if a powder (coloured) is given in the form of a lick the path taken is almost exclusively through the rumen, and the explanation that the arbitrary passage of powder in spoon-dosing is due to excited swallowing incident to forced administration, is a very feasible one.

Sojourn of Arsenic in the Stomach System.—The length of time during which the arsenic remains in the different compartments of the stomach obviously depends upon the path taken by the dose. Where the dose passes wholly into the rumen, elimination is comparatively slow. With No. 11387, 80 per cent. is recoverable eight hours after administration, and of this practically seven-eighths is still found in the rumen, into which it had been quantitatively injected. The seven per cent. in the reticulum presumably corresponds to material regorgitated from the rumen. Twenty per cent. of the dose has been passed on into the intestines and absorbed, but the amount held up in the abomasum is very small, and apparently corresponds to rumenal contents of low arsenical concentration still further diluted with gastric juice.

No. 11362, in which the dose has obviously passed for the most part into the rumen, is similar, and although 62 per cent. is still present in the rumenal contents eight hours after dosing (three hours after allowing access to food) the amount present in the abomasal contents is very small—2 mg. Of the total dose given, 35 per cent. has been passed on and absorbed.

It would therefore appear that whenever the dose passes wholly into the rumen the further passage of arsenic is gradual, and that the actual concentration in the abomasum, the habitat of the wireworm, is low. It is possible that in the hours before allowing access to food the arsenic in the abomasum may be a little higher than in the cases just considered, but both quantity and concentration must obviously be limited by the dilution effected in the large bulk of the rumenal contents. That the passage of arsenic from the rumen is

slow is also indicated by No. 9725, in which about a quarter of that given in the dose is still recoverable twenty-four hours after administration. Even three days after dosing, arsenic can still be detected in the rumen, although the small amount (2 mg.) found in the case of No. 10763 is not susceptible of quantitative interpretation owing to the fact that the original path of the dose is uncertain.

When the dose takes the rumenal route the worms in the abomasum are subjected to the prolonged influence of arsenic in very low concentration. If we take the average rumenal contents as about 2-3 kilos the 0.1 gm. soluble As_2O_3 in the dose corresponds to a concentration of .003-.005 per cent. But since the arsenite is in solution mainly in the mobile water of the rumenal contents the true aqueous concentration is somewhat higher. The further diluted and more fluid abomasal contents, however, would always be considerably below this figure. Thus in the case of No. 11387, eight hours after dosing, the concentration is actually .0016 per cent. (69 mg. in 4220 gm.), and in the fluid abomasal contents is .0008 per cent. (3 mg. in 375 gm.). It seems probably that whenever the dose goes *wholly* into the rumen the concentration in the abomasum rarely exceeds .001-.002 per cent. As_2O_3 at any time. That so low a concentration, even after prolonged action, is effective in destroying the wire-worms is not absolutely certain, but is highly probable. In case 11387 the wire-worms were not destroyed at the time of killing the sheep eight hours after dosing, but the duration of previous exposure to the arsenical concentration indicated at death (approximately .001 per cent.) is, of course, uncertain. On the other hand Veglia's observations, and the uniformly successful results of dosing on the large scale in the field, suggest that the wire-worms are destroyed irrespective of the path taken by the dose, and are, therefore, susceptible to slow intoxication by very low concentration of arsenic.

In general dosing practice, however, it seems safe to consider the main intoxication of the worms as being effected by the portion of the dose which reaches the abomasum direct, a secondary slow intoxication being then maintained by the gradual passage of the portion of the dose which first passed into the rumen. When the dose swallowed is passed mainly into the abomasum the arsenical concentration of the abomasal contents is relatively high, and the period during which the worms are under the influence of this higher concentration is quite considerable. With No. 8325 the greater part of the arsenic has obviously escaped the rumen, and about half of the total dose has been retained in the abomasum up to the time of killing two hours after administration. The concentration at the end of this time is still 54 mg. in 260 gm., or over .02 per cent. As_2O_3 —about twenty times higher than one would expect it to be if the dose had taken the rumenal route. No. 8133 is similar, and indicates that even four hours after dosing the arsenical concentration in the abomasum may still be high, .017 per cent., or 26 mg. in 150 gm., even although a large proportion (61 per cent.) of the total arsenic administered has by this time left the stomach system altogether. With No. 10676 the actual quantity of arsenic found in the stomach system five hours after dosing is higher (58 per cent.), though it happens that the concentration in the abomasum is lower owing to the greater volume of fluid contents. That a relatively high concentration may persist for at least eight hours

after dosing (three hours after feeding) is indicated by No. 10680, where 30 mg. are present in 255 gm. of abomasal contents. With No. 10868, also killed eight hours after dosing, both quantity and concentration are considerably lower. With No. 10576, 43 per cent. of the ingested arsenic is still in the rumen five hours after administration, a fact which suggests that about 50 per cent. of the dose originally found its way there. Of the remaining 50 per cent. almost the whole has passed on into the intestines, and only 3 mg. is present in the abomasum. Obviously in this case the abomasum has almost completely emptied itself into the duodenum at some period earlier than the fifth hour after dosing.

Although it is thus obvious that there is no very great constancy in behaviour and that the length of stay of arsenic reaching the abomasum is arbitrary, and conditioned by somewhat arbitrary contractions and relaxations of the duodenal sphincter, it is equally obvious that the concentration of arsenic in the abomasum generally remains fairly high for a good few hours after any considerable portion of the dose has arrived there, and that the worms are in general subjected to a fairly intense intoxication for anything from two to eight hours. It does not seem likely that the abomasum ever retains its original contents for much longer than eight hours, and as just indicated in the case of No. 10576 it may empty itself more or less completely within five hours, or, as indicated by No. 8325, pass on part of its contents (19 per cent. of the total arsenic administered) within two hours. After twelve hours (seven hours after access to food) we may, in general, safely regard any arsenic originally passed into the abomasum as having gone on into the intestine, and any arsenic still present as being derived from the rumen or washed out of lodgment in the reticulum. No. 8969 illustrates a case where a considerable amount of the powder had lodged in the reticulum, the surface of which showed greenish patches (copper arsenite) twenty-four hours after dosing.

With Nos. 10638 and 10753, killed twelve hours after dosing, and No. 10576 killed eight hours after dosing, a considerable proportion of the powder must have originally passed into the abomasum, but only a few milligrams remain there.

In two or three days we may normally regard all except traces of the administered arsenic as having left the stomach system altogether, whatever the channel taken by the dose initially.

Influence of Dietetic Treatment.—This unfortunately is not shown up by the limited data of Table I, and much further work would be required to illustrate it in numerical form.

In general one would expect the sojourn of the dose in the stomach system to be longer when the sheep are starved beforehand (sixteen to eighteen hours) and the stomach movements reduced to a minimum; and would expect the movements to be more active again shortly after allowing food, five hours after dosing. In dealing with large numbers of sheep this expectation may perhaps be realized, especially as regards the stay in the abomasum, but the limited available data do not establish it.

With No. 8133, 61 per cent. of the arsenic has passed out of the stomach system four hours after dosing in spite of the preliminary

starvation period and the withholding of food after dosing. The very low figure for the rumen indicates that the greater part of the dose must have gone direct to the abomasum, but only a quarter of it remains there. With No. 10676, 36 per cent. of the dose is still present in the abomasum five hours after dosing, and, although the data do not show it, it is quite possible that the abomasum would have emptied sooner if no preliminary starvation had been enforced. Nos. 10576 and 11362 were treated in the same way except for the starvation factor, but in both cases a large proportion of the arsenic passed into the rumen, and whatever fraction did originally reach the abomasum has in both cases passed further within eight hours. No very rigorous comparison is therefore possible, although the evidence, such as it is, rather suggests that any influence of the starvation period was obliterated within eight hours after dosing, or three hours after allowing access to food. With No. 10680, a duplicate of No. 10576, most of the arsenic passed into the abomasum direct, and in spite of the feeding five hours after dosing nearly one-third of the total arsenic administered is still there three hours later—i.e. eight hours after administration. In this case the giving of food has not had the effect of stimulating movement and emptying the abomasum—it is comparable with No. 8133, in which still less arsenic is present in the abomasum, although no food was given and the sheep was killed four hours sooner. No. 10868 is comparable with No. 10680 except for the withholding of food after dosing, but this procedure has not had the effect of prolonging the stay of the dose. The former shows 33 per cent. in the whole stomach system and only 16 per cent. in the abomasum, as against 41 per cent. and 30 per cent. respectively for the latter.

No. 9705, which was intended to show the effect of the administration of water after dosing, does not bring out the desired information owing to the fact that the bulk of the dose passed into the rumen—from which it would not be so easily washed out by water. But although on this account the absence of arsenic in the abomasum signifies little, especially in comparison with No. 11362, which was allowed food but no water, the very low bulk of the abomasal contents (30 gm.) suggests that the giving of water had the effect of stimulating the opening of the duodenal sphincter and of so emptying the abomasum; and that if the bulk of the dose had passed into the abomasum instead of the rumen it would have been hurried on to the duodenum by the giving of water. On the other hand the abnormally low abomasal contents in this case may have been fortuitous and quite unrelated to the stimulus of water intake.

At this point the results of Veglia's experiments upon the influence of short starvation periods upon rumination and upon the volume of the contents in the stomach system may be mentioned in the light of some of the data of Table I. According to Veglia's observations rumination was marked after a feed, but became more and more remittent as the starvation period lengthened, until after seventeen hours it practically ceased. At the same time the semi-solid contents of the rumen were not reduced to the extent which might have been expected, but maintained a minimum average bulk, and in some cases remained as high as 4.5 kilos after seventeen to twenty-four hours starvation. The contents of the rumen and reticulum in sheep killed two to four hours after feeding were in general not much greater than

in those killed after a day's starvation, and individual variation smothered all clear differences. The contents of the abomasum varied more, and, although in some cases the bulk after long starvation was suggested as lower than after short starvation, the differences were by no means so marked as might have been expected, but were, on the whole, of an arbitrary character. The extreme range in the abomasal contents was 25 c.c. to 940 c.c. for seventeen sheep killed two to four hours after feeding, and 55 c.c. to 360 c.c. for twenty-seven sheep killed after seventeen to twenty-four hours starvation. But this, of course, does not necessarily mean that the abomasal contents have remained stationary and unaltered during starvation—the abomasum may have emptied itself during the interval, and then have been recharged by passage of mobile material from the rumen, supplemented by gastric secretion. Although, therefore, the abomasal contents may often be considerable after twenty-four hours starvation it does not follow that any arsenic reaching the abomasum in the early period remains there for anything like twenty-four hours. In Table I we have data which show that the rate of passage of dissolved arsenic through the abomasum is not related to the amount of fluid present at any given moment. Thus with No. 10868, killed eight hours after dosing, only 33 per cent. of the dose remains in the whole stomach system. Of this, approximately 17 mg. remain in the rumen, reticulum, and omasum. 83 per cent. must, therefore, have passed into the abomasum at some time before killing—the greater part at the time of dosing. Of this 83 mg. only 16 mg. actually remain in the abomasum, the rest having passed on in solution into the intestines. Yet the residual bulk of abomasal contents is 525 c.c., and unless we make the absurd assumption that the original abomasal contents (at the time of dosing after seventeen hours starvation) exceeded 2 litres, we seem to have clear evidence that the abomasum has, at some time within eight hours, allowed the greater part of its contents to pass the duodenal sphincter and has then been supplemented with fresh material; fluid made up of mobile material from the rumen and of gastric secretion, since no fresh food was given.

All these considerations concerning the influence of dietetic treatment upon the rate of passage of arsenic through the abomasum are obviously somewhat speculative, and as already mentioned a much larger number of cases would have to be drawn into analytical consideration before quantitative data could be presented free from ambiguity. But although the direct evidence is meagre and ambiguous the fact that the mode of dietetic treatment has been found serviceable in practice on a very large scale is fair presumptive indirect evidence of the soundness of the teleological considerations which suggested its trial. Theiler had previously observed that sheep could safely tolerate larger doses of arsenic when water was withheld, and Veglia has found that the preliminary starvation period increases the general effectiveness of the dose in destroying wire-worms.

Passage of Arsenic through the Intestines.—The data of Table I indicate that arsenic is rapidly absorbed in the first intestine immediately after leaving the abomasum, and suggest that it is only in exceptional cases, usually accompanied by diarrhœa, that any notable quantity finds its way into the second intestine. The amount of arsenic found in the first intestine is always very small, and the arsenical concentration of the contents always low. The same applies

to the second intestine, except where the irritation set up in the first intestine has caused a rapid passage of its contents into the second. This has happened with Nos. 8969 and 10753, in which the arsenic found in the large intestine was very considerable (12-14 mg., or over 10 per cent. of the dose), and the consistency of the contents themselves more fluid than usual. Where such rushing through of arsenic takes place the amount of arsenic in the second intestine appears to *remain* fairly high, thus suggesting that actual absorption in this part of the tract is not rapid. The evidence of No. 8969, killed twenty-four hours after dosing, points in this direction, and even with No. 10753, killed twelve hours after dosing (second), it is highly improbable that the 14 mg. found was rushed from the abomasum through the first intestine and into the second intestine so soon before killing that no time for absorption was allowed.

As was to be expected on general grounds of analogy with the known absorption of other compounds, the first intestine, from the duodenum to the ileo-caecal valve, is indicated as the portion of the tract from which rapid absorption of arsenite takes place. The possibility of appreciable absorption from the abomasum itself has not yet been investigated, since somewhat troublesome operative procedure would be involved, but the fact that large amounts of arsenic may remain in the abomasum for a considerable time suggests that such absorption cannot be very marked. The proportion of the ingested dose which escapes intestinal absorption and is eliminated in the faeces, and the rate of elimination of absorbed arsenic through the urine, will be dealt with in more detail presently, but meanwhile it is obvious that absorption is rapid and fairly complete. With No. 8133, killed four hours after dosing, only 5 mg. of the 61 mg. passed on into the intestines are still present. Two milligrams have escaped into the second intestine, and 3 mg. are still in process of absorption in the first. The remainder has not only been absorbed, but, as shown by a test on the urine found in the bladder at post-mortem, has already been in part eliminated by the kidneys. It may be mentioned in passing that the first urine passed after dosing, even if micturation occurs within an hour, may sometimes contain appreciable amounts of arsenic, thus suggesting that elimination can be as rapid as absorption. In the case of No. 10868, 17 per cent. of the ingested arsenic had been eliminated by the time of death eight hours after dosing. In the case of No. 10641 the faeces collected up to the time of death only showed 1.5 mg. As_2O_3 , while in the intestines another 1.5 mg. could still be detected. Allowing for material still present in the rumen, approximately 96 per cent. of the administered dose had, therefore, been absorbed. Of this *at least* 34 per cent. was passed out in the urine of the first day, and *at least* 21 per cent. on the second. Absorption from the intestines *can*, therefore, be almost perfect, and elimination may be rapid. Actual rate of elimination will be dealt with in more detail, further on.

IV.—ARSENIC FIXED IN THE LIVER.

The data given in the last column of Table I were acquired chiefly with the object of providing quantitative figures under circumstances in which not only the amount of arsenic ingested is known, but also the extent of absorption and the period elapsing after ingestion. They

show the rapidity with which arsenic can appear fixed in the tissues (Nos. 8325 and 8133), and indicate quantities which can be fixed in the liver without necessarily having any particular significance from a toxicological point of view (Nos. 10680 and 10868).

In regard to the amount of arsenic which is present in the liver at various intervals after administration, there are considerable irregularities, which, however, can be readily explained on the natural assumption that the amount fixed depends upon the rate of absorption of the ingested dose—that absorbed arsenic is quickly fixed and as quickly eliminated again, the amount retained probably depending upon the concentration in the blood supply at any given moment.

In comparing No. 10638 with No. 10868, the figures for the stomach system suggest a similarity in rate and extent of absorption, yet the latter killed eight hours after dosing shows 3.6 mg. in the liver, and the former killed twelve hours after dosing shows only 1.1 mg. No. 9725, killed twenty-four hours after dosing, shows only half a milligram in the whole liver. No. 10641, killed forty-eight hours after dosing, shows a similar small amount (0.7 mg.). No. 10763, dosed twice at an interval of twenty-four hours, and killed for investigation three days after the second dose, shows only a trace of arsenic still retained in the liver. The greater part of the 240 mg. As_2O_3 , as soluble arsenite, had been absorbed, and considerably more than half of this had already been eliminated in the urine and faeces by the time of killing. There is little doubt that if the animal had been killed some time during the first day after the second dose, the liver would have shown at least 2-3 mg. instead of the mere fraction of a milligram found three days later. No. 11362 shows 2.3 mg. in the liver eight hours after dosing, although at this time less than 35 mg. had been absorbed from the alimentary system. The suggestion is that part of the dose was passed into the abomasum, thence more or less suddenly into the intestines a few hours later, and then rapidly absorbed, so flushing the liver and causing appreciable fixation. This same liver might possibly have shown less arsenic if the animal had been killed a few hours later (as in the case of No. 10638), although the continued slow passage of the rumenal contents (containing 62 mg.) might tend to maintain it at the higher level. In the case of No. 11387, where the dose is known to have been quantitatively passed into the rumen (injected) the passage of the dose must of necessity have been gradual, and although about 20 mg. appear to have been absorbed, only traces appear in the liver eight hours after administration. No. 10753, killed twelve hours after a second dose, shows 1.2 mg. in the liver. The 27 mg. still in the rumen may be derived from the first or second dose, but the abomasum contains little or no arsenic, and the greater part of the ingested dose of the second administration appears to have been absorbed early. This liver might easily have shown a higher figure if the animal had been killed for examination a few hours sooner. There is, of course, no definite hour after ingestion at which the liver reaches its maximum of arsenic, since this is presumably conditioned by a rate of absorption which varies with the path taken by the dose and the period of sojourn in the different compartments of the stomach. If the dose takes the rumenal path exclusively, absorption is naturally gradual, and no very high figure for arsenic in the liver would be expected at any time. If the bulk of the dose goes

direct to the abomasum, and is fairly quickly passed on into the intestines, an appreciable figure for the liver would be anticipated a few hours later, rising as long as rapid absorption was going on, and falling again a few hours after absorption was over. The liver certainly fixes arsenic more easily than the other tissues, but it appears to get rid of it again with equal ease. Analyses of other tissues (kidneys, spleen, muscles, etc.) were not made in all cases, owing to pressure of other work, but in the few which were carried out the proportion of arsenic in the liver at any short interval (eight to forty-eight hours) after dosing was invariably much higher than in any of the other organs. Even when the liver contained nearly 1 mg. As_2O_3 per 100 gm. of fresh tissue, the kidneys contained considerably less, the spleen still less, and the blood and muscles minute amounts difficult to determine with any accuracy.

The data available in this direction for the sheep of Table I are not complete enough or certain enough for numerical discussion, but a case showing the distribution of arsenic in an animal which died of arsenical poisoning between four and five hours after drenching with 100 c.c. of a solution of sodium of arsenite equivalent to 0.512 gm. As_2O_3 , is of considerable interest. The protocols are given in Table II. The weights of the contents of the alimentary system are given approximately, the residual contents left after rough removal being washed out with water, and the total arsenic calculated from aliquots taken for analysis after making up to definite weight. The data " As_2O_3 per 100 gm." are then calculated from the first approximate weighing of contents. The figure for the quantity of blood is gauged on the basis of total blood volume equivalent to about 8 per cent. of the body weight.

Table II.

Sheep No. 9724. Weight, 40 kilos. Drenched 9 a.m. with 100 c.c. of sodium arsenite solution, equivalent to 512 mg. As_2O_3 . Died between 1 p.m. and 2 p.m., i.e. 4-5 hours after dosing.

No faeces or urine passed between dosing and death.

Analysed.	Weight in gm.	As_2O_3 Total in mg.	As_2O_3 Mg. per 100 gm.
Rumen Contents.....	1,490	125	8.4
Reticulum Contents.....	200	13.6	6.8
Abomasum Contents.....	760	275	36.2
Omasum Contents.....	15	2.6	17
First Intestine.....	250	2.0	0.8
Second Intestine.....	300	trace	trace
Liver.....	475	23	4.8
Spleen.....	54	0.32	0.6
Kidneys.....	80	1.7	2.1
Blood.....	(3,200)	11.5	0.36
Muscle (gluteus).....	sample	[10.0 (?)]	[.05 (?)]
Urine in the Bladder.....	Lost but small in amount	—	—

Total arsenic accounted for in parts analysed, about 465 mg. or 91 per cent. of the dose.

Of the total arsenic administered, .465 gm. out of .512 gm., or 91 per cent. is accounted for. Of the remaining

9 per cent., about 5 per cent. may still be due to error, since in dealing with small amounts of arsenic in large bulk of material it is difficult to state results closer than ± 5 per cent. From .418 grm. to .439 grm. still remains in the alimentary tract, almost wholly in the stomach system, while 73mg. to 94 mg. has been absorbed. It is a little surprising that this should occasion death, since, as will be shown later, it is usually quite safe to inject 100 mg. straight into the blood stream by the jugular vein. The suggested possibility that arsenic rapidly absorbed through the intestines and carried by the portal vein straight to the liver and extensively fixed there, is more rapidly fatal than arsenic injected into the jugular and largely distributed over the massive muscular tissues, is too speculative for discussion without further data. In this particular case the sheep may have been unduly sensitive to arsenic. Whether the extensive gastric and intestinal hyperaemia occasioned by the ingestion of so large a dose of soluble arsenite is a serious contributory cause of death, it is impossible to say.

It is, however, interesting to compare this Table II with Table X (discussed later), in which the sheep was killed for analysis of tissues half an hour after injection of 100 mg. As_2O_3 as sodium arsenite. Although approximately the same amount of arsenic may be assumed to have reached the blood stream in both cases, the proportion of arsenic fixed in the liver is twice as high in Table II as in Table X. This is probably due to the fact that all the arsenic absorbed from the intestine by the blood has to pass by the portal vein through the liver before it reaches the general circulation, and that the opportunity for fixation by the liver is, therefore, exceptionally great; whereas, arsenic injected into the jugular vein is distributed direct from the heart over the whole body, so encouraging a more appreciable fixation in the muscular tissues. Beyond this we do not care to contrast the two tables without further experimentation and verification. The higher value for the blood concentration and the much lower value for muscle in Table II may not be quite accurate, since the analyses were carried out early in our work, when less attention was paid to minute quantities of arsenic in the muscles than was paid in the case of the later results. There is no obvious reason why the blood values should differ so much in the two tables, and although one would expect a lower value for the muscles in Table II owing to the "sieve" action of the liver on the portal blood, one would hardly expect the difference to be so sharp as is actually recorded. At a later date we hope to return specifically to this question of the difference in distribution in the tissues after intestinal absorption and intravenous injection, but meanwhile merely wish to emphasize the fact that rapid absorption of arsenic from the intestine may result in a figure for liver tissue higher than that produced by intravenous injection of an amount of arsenic similar to that absorbed.

The flooding of the liver with arsenic is particularly interesting in comparison with the small amounts found in the other tissues, and the amount found in the liver of sheep No. 10868, Table I, eight hours after administering a perfectly safe dose of arsenite, need therefore occasion no surprise.

It may be noted that the arsenic in the kidneys is high "per 100

gram.," although low in absolute amount—2.1 mg. per 100 gm. or 1.7 mg. in all, against 4.8 per 100 gm. or 23 mg. total for the liver.

In regard to the amount of arsenic in the liver which can be regarded as of toxicological importance in diagnosis of arsenical poisoning, the present data are meagre, but, nevertheless, of some interest. No. 10680 and 10868 of Table I show quite clearly that no particular significance need necessarily attach to quantities in the region of 1 mg. per 100 gm. of liver tissue, while the data of Table II show that a quantity in the region of 5 mg. per 100 gm. may correspond to an acute case of poisoning, in which death ensued within five hours. From our miscellaneous analytical records of clearly established cases of arsenical poisoning in various animals we have no doubt whatever that whenever a liver reaches this higher figure it is perfectly safe to diagnose arsenical poisoning, irrespective of suggested other cause of mortality. Indeed we should feel fairly confident in reporting an amount such as 2 mg. per 100 gm. as fatal arsenical intoxication, in absence of other evidence. Horses have come under observation (crops destroyed by experimental poisoning), in which the arsenic in the liver in fatal cases was below 1 mg. As_2O_3 per 100 gm., although in such cases death was not rapid, but occurred thirty-six to seventy-two hours after dosing. But the data now presented for sheep, dosed with quantities of arsenite demonstrated as perfectly safe by the records of thousands of sheep in the field, show quite clearly that in a case of suspected poisoning it *might* be quite erroneous to deduce death from arsenical intoxication on the evidence of 1 mg. As_2O_3 per 100 gm. of liver—or on the evidence of 2 mg. per 100 gm. of rumenal contents. A qualitative test, as sometimes practised, is of very little value. Whether 1 mg. per 100 gm. of liver tissue could always be regarded as definitely pointing to arsenical intoxication if the animal is known to have had no possible access to arsenic for forty-eight hours before death, is not quite certain, but we are inclined to think it could.

It may be asked why it is necessary to report upon a quantitative basis at all—why the mere presence of appreciable quantities of arsenic, as indicated by a good qualitative test, is not sufficient. The reason is that in countries such as South Africa, where dipping of live stock in arsenical baths and dosing with arsenical preparations is extensively practised, arsenic may be frequently be found in relieta without necessarily meaning anything at all. In human toxicology the mere presence of arsenic in relieta is usually suspicious, and circumstantial evidence is often easier to sift, but with live stock the problem is complicated by the fact that amounts of arsenic which show up well by the Gutzeit test may have been derived by safe medicinal treatment or accidental swallowing of a small, harmless quantity of arsenical dip. Indeed, in regularly dipped animals an organ such as the liver may contain easily detected quantities of arsenic derived by skin absorption. Such traces mean nothing. We have frequently had cases to deal with in which animals died shortly after dipping, and in which the circumstantial evidence suggested poisoning to the owner. But, unfortunately, circumstantial evidence of such a kind is seriously weakened by the fact that animals are habitually dipped at such short intervals that they are as likely to die from extraneous causes just after dipping as at any other time. In some such cases analysis of ingesta or organs shows so little arsenic that arsenical intoxication

can be ruled out. In other instances the amount of arsenic is quite appreciable, but not definitive, and the cause of death becomes difficult to explain unless intercurrent disease can be diagnosed or some valid concomitant factor brought to light. An instance in point may be cited, in which seven out of about forty cattle died within twenty-four hours of dipping. Relicta of two of these were examined. The liver of one contained 0.25 mg. As_2O_3 per 100 grm., and the other 0.15 mg. In both cases arsenic could also be detected in the rumen, indicating that at least small quantities had got in through the mouth. Without extensive quantitative data concerning authentic poisoning cases, and the limits of safe fortuitous occurrence of arsenic, the analyst might be tempted to accept arsenical poisoning as proven. But as it happened, blood-smear examination and the subsequent history of the herd showed quite unmistakably that the deaths were due to anthrax. In this case the small amount of arsenic ingested by at least two of the cattle was merely an accidental concomitant. Usually, however, so simple an alternative explanation does not crop up, and the true cause of death may be left in obscurity.

Cases in which a few sheep succumb after dipping a flock are also not uncommon. If the dip happens to be an arsenical one, the owner is apt to attribute his loss to poisoning. In such cases arsenic is frequently detectable in the relict, and the urine in the bladder at death may contain appreciable quantities of arsenic derived by skin absorption or by accidental swallowing of quantities of no toxicological importance—only one-tenth, perhaps, of a minimal toxic dose. The fatalities might have occurred if the sheep had been dipped in any non-poisonous fluid; may in some cases even be traceable to traumatic pneumonia. The analytical data might then be irrelevant, and unless the analyst were careful, his report might be very misleading.

Cases in which the question arises as to whether the arsenic found by the analyst can or cannot account for death are not always of much importance; but they may be, especially if the question of compensation be involved, or the problem of possible malicious poisoning be raised.

Hitherto we have been inclined to regard 0.5 mg. As_2O_3 per 100 gms. of liver tissue as indicating intoxication, but Sheep Nos. 10680 and 10868 upset any empirical generalization. On the other hand, it would be fallacious to disregard a figure such as 0.9 mg. (No. 10680 expressed per 100 gm.) as beneath suspicion. On the contrary, since quite a number of well established poisoning cases (in cattle and horses at least) have shown no more, the analyst would have good justification for deducing intoxication unless some other more plausible explanation for death could be brought to light. But the instance serves to show the difficulty of drawing conclusions where the circumstantial evidence is imperfect. If Sheep Nos. 10680 and 10868 had died in the field under treatment for wire-worm, an analyst, without being aware of the circumstances, might well report arsenical poisoning—and be wrong. A veterinary surgeon might, on post-mortem evidence, report death as due to the wire-worm infection itself, and with a greater chance of being right. Incidentally it may be remarked that some sheep owners tend to regard losses “after dosing” as losses “due to dosing”—quite erroneously.

Whether it would be possible to fix diagnostic criteria "negligible," "suspicious," and "fatal" for an organ such as the liver, in such a way as to make them of definite value for the generality of unknown poisoning cases, is very doubtful. Data in this direction are of course of value as aids to interpretation and safeguards against rash deductions, but their significance is limited. So far as our own miscellaneous evidence goes, 0.1–0.2 mg. As_2O_3 per 100 grms. of liver tissue is negligible and incapable of interpretation; 0.4–0.8 mg. would be suspicious, depending upon the circumstantial evidence; above 1 mg. would be highly suspicious under most circumstances; and 2 mg. or more (per 100 gm.) would be an almost certain indication of fatal intoxication—could account for death irrespective of possible other causes. The matter is complicated by the fact that a low figure (such as 0.5 mg. per 100 gm. of liver tissue) might indicate nothing in particular in the case of rapid absorption of a non-toxic dose, but possibly indicate fatal intoxication in a case of delayed poisoning. This is in conflict with the view expressed by Lander (Veterinary Toxicology, 1912 ed., p. 37) that in "acute cases arsenic is speedily found in the liver, *although in less quantity than in sub-acute or slow poisoning cases.*" Within our experience, now covering a very considerable number of cases, the arsenic has been found to reach the liver not only speedily but in *much larger amount* in acute than in sub-acute cases. In Lander's instances of cows poisoned by dip, Case I died within twenty-four hours, and only showed a trace of arsenic in the liver, although it showed a very large amount in the rumen— $\frac{1}{8}$ th grain per oz., equivalent to 38 mgs. per 100 gm. of contents, which would work out at something in the region of 10 gm. in the whole rumen. Case II died after five days, and Case III after seven days, showing $\frac{1}{9000}$ th and $\frac{1}{5000}$ th grain per oz., equivalent to 0.25 mg. and 0.47 mg. per 100 gm. of liver tissue, respectively. A glance at Table I shows that, for sheep at any rate, such quantities can appear in the liver within a few hours after ingestion of perfectly safe doses of arsenite. In three cases of cattle, also poisoned by dip, recently investigated in this laboratory, the results ran as follows:—

Case.	Rumen.	Intestines.	Liver.	Kidneys.	Period of Illness.
	Mg. per 100 gram.	Mg. per 100 gram.	Mg. per 100 gram.	Mg. per 100 gram.	
A.....	4.5	0.2	3.5	1.9	Less than one day.
B.....	0.34	0.3	2.2	1.3	2 days
C.....	0.36	0.4	1.3	1.1	2 days.

Here all three livers show several times as much arsenic as any of Lander's cases, and the highest amount is shown by the animal which succumbed first. Lander's Case III, in which $\frac{1}{24}$ th grain per oz. or 9.6 mg. per 100 gm. was found in the rumen contents is very interesting. On general grounds one would expect the rumen to be nearly free of arsenic within a week after ingestion of it in *soluble*

form (dip), unless no food was taken during the whole period of illness. It is interesting to note that in Lander's cases the evidence of the rumen contents is the stronger, whereas in our own the liver figures suggest fatal intoxication, while the rumen contents of B and C do not; 0.34 mg. per 100 grm. might only mean 0.1 to 0.15 grm. in the whole rumen, a quantity which is occasionally swallowed by cattle during dipping with no ill effects whatever. The figure 2.2 mg. per 100 grm. of liver tissue does, however, explain the fatality and the suggestion is that the greater part of the ingested dip had been absorbed by the time death occurred. Conversely in Lander's Case II 0.25 mg. As_2O_3 per 100 grm. of liver tissue, taken alone, might possibly only mean that the animal had been dipped but not necessarily that it had swallowed a *toxic* amount; whereas the figure 1/30th grain per oz. of rumen contents, or about 8 mg. per 100 grm., suggest a dangerous total of over 3 grm. (soluble) in the whole rumen. A combination of analyses of stomach contents and liver tissue may therefore be easier to interpret than either alone.

This discussion of the interpretation of analytical data in poisoning cases is rather an off-shoot from the main conclusions of Table I, and is admittedly not particularly illuminating—raises the problem rather than solves it. It is hoped, however, to devote special consideration to it at a later date, when a further accumulation of miscellaneous data on well-defined poisoning cases becomes available. Meanwhile information bearing upon quantities of arsenic which can occur in the alimentary system, at various intervals after the ingestion of non-toxic doses, is of general scientific value, and since the available information in this direction is so scanty no apology is needed for the limited additions already offered in Table I.

V.—ELIMINATION OF INGESTED ARSENIC IN THE URINE AND FAECES.

In discussing Table I it has already been mentioned that ingested arsenite may appear very rapidly in the urine, and that a very considerable proportion of the dose may be eliminated within twenty-four hours; also that the moiety passed out in the faeces may be small in comparison with that passed out in the urine. But the data offered were limited by the fact that the main purpose of the experiments involved the killing of the sheep, and it was therefore considered of interest to carry out a little systematic work specially dealing with the rate of elimination. The information was primarily wanted in connection with sheep in order that our knowledge of the fate of arsenic in dosing for wire-worm should be as complete as possible, but since so little accurately detailed work, in which the ingested dose can be more or less quantitatively accounted for, is recorded for any class of animal the results should have a wider scientific value.

The male sheep is a convenient experimental animal and offers little difficulty in the way of collection of faeces and urine. During experiment the sheep was confined in a wooden metabolism crate with hands passed loosely under the belly to act as a sling and support the animal when it got tired of standing. Faeces were collected in a linen droppings-bag, and a thick glass beaker (about 1 litre capacity) contained in a well-fitting leather cradle was strapped over the penis for the collection of urine, the wool being clipped short over the area

covered by the beaker. Faeces were taken for analysis at twenty-four hour periods, and the urine removed at intervals throughout the twenty-four hours according to requirements. The sheep is an animal which micturates frequently, and by keeping close watch on the glass collecting beaker it is possible to secure samples sufficiently often to determine the rate of elimination of any constituent of the urine with a very fair degree of accuracy—on the natural assumption, of course, that the bladder is emptied at each act of micturation. In the data which follow, the rate of elimination of ingested arsenic was determined in this way, although it was not considered necessary in all cases to mount guards on the beaker and carry out analyses on each separate passage of urine. In some cases the sheep were kept under constant observation and time of micturation recorded to within a few minutes of the truth. In other cases urine was collected by periodic visits to the crate, sufficiently close to give approximate correspondence between sample and time of passage. Close watch on the animal is tedious and time-consuming, and the possibilities of a device such as ignoring the actual moment of micturation and using the ratio $\text{As}_2\text{O}_3 : \text{Creatinine} + \text{Creatine}$ as a rough measure of the rate of arsenic elimination was therefore tested. Such a procedure would of course presuppose that the output of “Creatinine + Creatine” ($k + k'$ throughout the ensuing text) is fairly constant, but on trial this was found to be much less regular than had been anticipated; much less so than it is in the human subject. The idea was therefore abandoned, the error in the use of such a ratio being too high for the purpose of the present investigation. Apart, however, from the uncertainty of $k + k'$ as an endogenous constant upon which to tag an exogenous variable, the elimination of creatinine and creatine itself was considered worth observing; as also the influence of arsenic, if any, upon the creatine output. No detailed data upon creatinine and creatine elimination seem to be recorded for the sheep, and since the estimations involved were simple in comparison with the trouble in keeping the sheep under constant observation and following out the arsenic elimination, they were in most cases carried out.

The diet, consisting of green fodder (usually lucerne), veld hay, and maize, was constant in each experiment, and more or less the same throughout the whole series. Although no special attention was paid to voluntary variations in the intake, there is little doubt that the intake was fairly constant except where otherwise specified. The variations in the $k + k'$ figures can therefore probably be treated as endogenous and more or less independent of the exogenous metabolism. The variations in the output from day to day or from hour to hour, and the variations in the relative proportion of creatine to creatinine are indicated in the tables, and will, it is hoped, be of provisional general interest. At a later date we hope to deal briefly again with this question and offer a few data on output under varying dietary conditions, uncomplicated by arsenical treatment.

Tables III and IV represent duplicate experiments on the same sheep, carried out at an interval of about a month, a dose of sodium arsenite equivalent to 0.1 gm. As_2O_3 being administered in 100 c.c. of water. This amount is equivalent to the arsenite contained in a single dose of wire-worm remedy for an adult sheep. Table III shows the daily output of arsenic in urine and faeces for ten days. Table IV

records urinary elimination at shorter intervals during the first few days, and also gives figures for the creatinine and creatine output.

Table III.

Adult sheep No. 11010. Weight 65 lb., but in very poor condition. In good condition later, weighed 81 lb. Starved 17 hours; dosed with 125 mg. sodium arsenite in water, equivalent to 100 mg. As_2O_3 . Given green food 5 hours later, but no water. Next morning, i.e. 24 hours after dosing, given food and water *ad lib*.

PERIOD AFTER DOSING.	URINE.			FÆCES.		
	Quantity.	As_2O_3 per 100 c.c.	As_2O_3 Total.	Quantity.	As_2O_3 per 100 grm.	As_2O_3 Total.
	c.c.	mg.	mg.	grm.	mg.	mg.
First day (24 hours).....	130	19.2	24.9	250	0.52	1.3
Second day.....	930	2.57	23.9	80	2.20	1.8
Third day.....	500	2.70	13.5	218	2.00	4.4
Fourth day.....	450	0.96	4.3	271	1.40	3.9
Fifth day.....	300	0.80	2.4	390	0.52	2.0
Sixth day.....	550	0.36	2.0	370	0.16	0.6
Seventh day.....	650	0.37	2.4	330	0.06	0.2
Eighth day.....	300	0.23	0.7	360	trace	trace
Ninth day.....	120	0.33	0.4	—	—	—
Tenth day.....	320	0.12	0.4	—	—	—
TOTAL.....		—	74.9	—	—	14.2

Total As_2O_3 recovered = 89.1 mg., or 89 ± 5 per cent. of the dose.

TABLE IV :

ENSUING PAGE 505.

Table IV.

Sheep No. 11010. Weight 65 lb., but in poor condition: in good condition a few months later weighed 81 lb. Starved 17 hours, drenched with 0.125 sodium arsenite, equivalent to 100 mg. As_2O_3 , in water at 9 a.m., 2nd July, 1917. Offered green food 2 p.m.-4 p.m. but no water. From 9 a.m. next morning onwards, food and water *ad lib*. Urine quantities only approximately accurate, since the round figures given usually include a few c.c. of water used for rinsing out the collecting beaker. Periods accurate by observation of time of micturition to within $\frac{1}{4}$ to $\frac{1}{2}$ hour for short periods and $\frac{1}{2}$ to 1 hour for long periods. In some cases analyses were carried out on single passages of urine, in others two or more passages were combined.

TIME	COLLECTION PERIOD.	ELIMINATION IN URINE.							IN FAECES.					
		Quant- ity.	Creatinine k.	Creatine k'.	k + k'.	k' as per- centage of k + k'.	k + k' per Hour.	As ₂ O ₃ per 100 c.c.	As ₂ O ₃ Total.	As ₂ O ₃ calculated per Hour.	Mg. As ₂ O ₃ per Gm.k + k'	Quan- tity.	As ₂ O ₃ per 100 grm.	Ass ₂ O ₃ Total.
1st day. .	9. 0 a.m.-11. 0 a.m.	c.c. (100)	grm. 0.020	grm. 0.066	grm. 0.033	30	mg. trace	mg. trace	mg. —	mg. —	<1	grm.	mg.	mg.
	11. 0 a.m.-4. 0 p.m.	95	0.096	0.153	0.249	37	6.0	5.7	1.12	37	43			
	4. 0 p.m.-9. 0 a.m.	350	0.311	0.282	0.593	47	7.2	25.2	1.43	43				
	TOTAL.....	595	0.453	0.359	0.812	45	(6.3)	30.9	—	—	—	110	0.21	0.36
2nd day. .	9. 0 a.m.-11.45 a.m.	135	0.090	0.018	0.108	17	3.5	4.7	1.1	43				
	11.45 a.m.-4.30 p.m.	185	0.101	0.095	0.196	48	4.0	7.4	1.56	38				
	4.30 p.m.-9. 0 a.m.	450	0.320	0.287	0.607	47	3.2	14.5	0.88	24				
	TOTAL.....	770	0.511	0.400	0.911	44	(3.4)	26.6	—	—	—	65	2.4	1.6
3rd day. .	9. 0 a.m.-4.30 p.m.	155	0.155	0.153	0.308	50	2.2	3.4	0.45	11				
	4.30 p.m.-9. 0 a.m.	230	0.328	0.273	0.601	45	2.1	4.8	0.29	8				
	TOTAL.....	385	0.483	0.426	0.909	47	(2.1)	8.2	—	—	—	300	1.1	3.3
4th day. .	First passage.....	60	0.096	0.080	0.176	45	0.95	0.57	—	3.2				
	Remainder.....	200	0.305	0.244	0.549	44	0.65	1.30	—	2.1				
	TOTAL.....	260	0.401	0.324	0.725	45	(0.73)	1.90	0.08	(2.6)		135	0.95	1.3
5th day. .	TOTAL.....	500	0.391	0.282	0.673	42	0.40	2.0	0.08	3		180	0.57	1.0
	TOTAL.....	1,100	0.400	0.326	0.726	45	0.030	3.1	0.13	4.3		170	0.27	0.46
	TOTAL.....	1,150	0.420	0.283	0.703	40	0.029	2.4	0.10	3.4		120	0.10	0.12
6th day. .	TOTAL.....	750	0.410	0.285	0.695	41	0.10	0.75	0.03	1.1		200	trace	trace
	TOTAL.....	640	0.390	0.282	0.672	42	0.10	0.64	0.03	<1				
	TOTAL.....													
Total As ₂ O ₃ recovered in 9 days = 84.6 mg. = 85 ± 5 per cent. of the dose.....														76.5
														8.1

A week later, i.e. sixteen days after dosing, arsenic could still be detected in the urine by delicate colorimetric testing, but only in traces corresponding to about one-hundredth of a milligram per 100 c.c.

The figures of Tables III and IV explain themselves and little discussion is required. By the end of the ninth or tenth day after dosing, arsenic had disappeared altogether from the faeces and the amount in the urine had fallen so low that it was not considered worth while continuing the analyses. Presumably a mere fraction of a milligram continued to be excreted daily for some time afterwards. As indicated at the foot of Table IV, arsenic could still be detected in minute amount a week after discontinuing the experiment, or sixteen days after dosing.

In Table III 89 mg., or 89 per cent. of the arsenic administered, is accounted for, of which 75 mg. appear in the urine and 14 mg. in the faeces. In Table IV the recovery is approximately 77 mg. in the urine and only 8 mg. in the faeces. Owing to the difficulty of accurately determining small amounts of arsenic, and in spite of correction of titration results by careful control experiments using known amounts of arsenic-free urine and faeces with known amounts of added arsenic, a possible error of one-twentieth either way must still be allowed in interpreting the results. The probabilities are that the data err on the low side rather than on the high side. The real amount accounted for must therefore be allowed to run as high as 90 to 94 per cent., or possibly (less likely) as low as 80 to 85 per cent. of the dose administered. The remaining 11 mg. (6 mg. to 16 mg.) in Table III and 15 mg. (possibly 20 mg., but probably only 10 mg.) in Table IV appear to be retained, presumably scattered as exceedingly minute amounts fixed in the tissues. The absolute amount, however, is so small in relation to the body weight of the sheep that it was not considered worth while killing the animal and attempting to determine the distribution. Presumably such small residual amounts are eliminated gradually in urine, faeces, perspiration, etc., over a period of several weeks, but in any case they are of little physiological consequence. The interesting thing is the relative proportions excreted in urine and faeces respectively, and the rate of elimination. By far the greater part of the ingested dose appears in the urine and only one-tenth to one-fifth as much in the faeces. Of the total arsenic eliminated in the urine two-thirds to three-quarters is passed out in the first forty-eight hours. The fall on the third day is marked, and thereafter only a few milligrams are eliminated per day, dwindling down to less than a milligram on the eighth day. In the faeces a little arsenic appears on the first day, corresponding presumably to unabsorbed residue of part of the dose which had originally gone direct to the abomasum and thence quickly into the intestines. The highest amount in the faeces appears on the third day in both cases—4.4 mg. and 3.3 mg. respectively. Thereafter the amount excreted tails off, and by the seventh or eighth day the last of the contents of the rumen at the time of dosing appear to have passed out as faeces, and the arsenic disappears.

From Table IV it may be noted that the first urine passed, corresponding to elimination for the first two hours after dosing, contains practically no arsenic. Absorption and elimination of the dose is not yet properly under way. By 4 o'clock in the afternoon, however, seven hours after dosing, as much as 5.7 mg. had appeared. Within the next seventeen hours over 25 mg. comes out in the urine, bringing the day's output up to 31 per cent. of the dose.

From the column headed " As_2O_3 per hour" it is seen that the

rate of excretion begins to rise after the first few hours, increases for about twenty-four hours, and then begins to slow down again. Between 11 a.m. and 4 p.m. on the first day, elimination in the urine averages 1.12 mg. per hour. During the night it averages 1.48 mg. The figure 1.71 mg. per hour for the period 9 a.m.-11.45 a.m. of the second day, shows that the rate of excretion is still increasing. Within the ensuing few hours it begins to fall again, being 1.56 mg. for the afternoon sample and .88 mg. for the overnight passage. By the morning of the third day it is down to .45 mg. per hour, which falls to .29 mg. overnight. Thereafter the rate is irregular but very slow.

Table V gives a similar set of data obtained on a different animal, the dose in this case being in powder form (wire-worm remedy) and administered with a spoon exactly as in dosing against wire-worm. The amount of arsenite administered is a little higher than in the preceding cases, being equivalent to 120 mg. As_2O_3 (150 mg. sodium arsenite) instead of 100 mg.

TABLE V:

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Table I.

Sheep No. 9724. Weight 80 lb., in moderately good condition. Starved 17 hours. Dosed 9 a.m. with wire-worm mixture, equivalent to 150 mg. sodium arsenite = 120 mg. As_2O_3 . Allowed green food 5 hours after dosing (2 p.m.-4 p.m.) and both food and water *ad lib.* from 9 a.m. next morning onwards. Urine quantities approximate—made up to figures given, with water used to rinse out the collecting vessel. Periods approximately accurate by observation of the time of micturition by frequent visits to the crate.

TIME.	COLLECTION PERIOD.	ELIMINATION IN URINE.										IN FAECES.		
		Quantity.	Creatinine k.	Creatine k.	k + k'.	k' as Per- centage of k + k'.	k + k' per Hour.	As ₂ O ₃ per 100 c.c.	As ₂ O ₃ Total.	As ₂ O ₃ calculated per Hour.	Mg. As ₂ O ₃ per Grm.k + k'.	Quant- ity.	As ₂ O ₃ per 100 grm.	As ₂ O ₃ Total.
1st day....	9. 0 a.m.- 5. 0 p.m.	c.c.	gm.	gm.	gm.		gm.	mg.	mg.	mg.		gm.	mg.	mg.
	5. 0 p.m.- 9. 0 a.m.	45	0.163	0.061	0.224	27	0.028	18.4	8.3	1.04	37			
		75	0.301	0.091	0.392	23	0.025	52.5	39.4	2.46	101			
	TOTAL.....	120	0.464	0.152	0.616	25	(0.026)	(39.7)	47.7	—	—	80	trace	trace
2nd day....	TOTAL.....	135	0.508	0.180	0.688	26	0.029	25.8	34.9	1.45	51	193	2.2	4.2
3rd day...	9. 0 a.m.- 2. 0 p.m.	75	0.206	0.024	0.230	11	0.046	5.5	4.1	0.82	18			
	2. 0 p.m.- 9. 0 a.m.	140	0.449	0.147	0.596	25	0.031	3.6	5.1	0.27	9			
	TOTAL.....	215	0.655	0.171	0.826	21	(0.034)	(4.3)	9.2	—	—	35	3.1	1.1
4th day...	9. 0 a.m.-12.30 p.m.	60	0.163	0.063	0.226	27	0.065	1.5	0.9	0.26	4			
	12.30 p.m.- 5. 0 p.m.	90	0.194	0.074	0.268	28	0.060	1.3	1.2	0.27	4			
	5. 0 p.m.- 9. 0 a.m.	140	0.440	0.227	0.667	35	0.042	1.4	2.0	0.13	3			
	TOTAL.....	290	0.797	0.364	1.161	31	(0.048)	(1.4)	4.1	—	—	120	2.5	3.0
5th day...	TOTAL.....	200	0.362	0.292	0.654	44	0.027	0.65	1.3	0.05	2	105	1.4	1.5
6th day...	TOTAL.....	120	0.454	0.12	0.626	27	0.026	0.42	0.5	0.02	<1	190	0.53	1.0
TOTAL.....											97.7	10.8		

Total As_2O_3 recovered in 6 days = 108.5 mg. = 90 ± 5 per cent. of the dose.

The results are much the same as before. 90 per cent. of the dose administered is recovered within six days, leaving 11 mg. (6 mg.-16 mg.) unaccounted for, and presumably retained temporarily in the tissues. Of this 90 per cent. eliminated, 81 per cent. appears in the urine and 9 per cent. in the faeces. Of the amount excreted in the faeces the highest figure appears on the second day, although the highest figure "per 100 grm." occurs on the third day. Part of the faeces passed on the fourth day should doubtless have been evacuated on the day before.

In the urine 47.7 mg., or 40 per cent. of the dose, appears in the first twenty-four hours, and 34.9 mg., or another 30 per cent., of the dose appears in the second twenty-four hours. Elimination is therefore rapid. Within the first eight hours after dosing the rate of excretion rises rapidly, averaging about 1 mg. per hour, but probably not being far off 2 mg. per hour at the eighth hour. The overnight elimination averages $2\frac{1}{2}$ mg. per hour. On the second day the average is $1\frac{1}{2}$ mg. per hour, which falls to .82 mg. on the morning of the third day, and to .27 mg. overnight. By the sixth day the rate is down to one-fiftieth of a milligram per hour, or half a milligram in the whole twenty-four hours' output. The traces eliminated during the ensuing week are not recorded quantitatively.

Table VI is interesting as showing the elimination of higher doses of arsenite, approaching perilously near the average dangerous dose. This animal (No. 9545) received three times the wire-worm dose for adult sheep without being any the worse for it. Such an amount might prove fatal in some cases, and could, therefore, not be advised therapeutically, although in the majority of cases it would probably prove safe. Much would depend upon the path taken by the dose, i.e. whether it passed wholly into the rumen, from which it would be more slowly absorbed, or whether it passed straight into the abomasum and thence rapidly into the intestines for speedy absorption.

TABLE VI:

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Table VI.

Sheep No. 9545. Dosed with 0.375 grm. sodium arsenite, equivalent to 300 mg. As_2O_3 in cachet form 9 a.m., 2nd October, 1917. No dietetic treatment—food and water as usual the whole time. Collection periods approximately accurate by observation of the periods of micturition. Quantities of urine approximately accurate, being made up to the figures given with a few c.c. of water used to rinse out the collecting vessel.

TIME.	COLLECTION PERIOD.	ELIMINATION IN URINE.										IN FAECES.		
		Quantity.	Creatinine k.	Creatine k.	k + k'.	k' as Per- centage of k + k'.	k + k' per Hour.	As_2O_3 per 100 c.c.	As_2O_3 Total.	As_2O_3 calculated per Hour.	Mg. As_2O_3 p.p.i. Grm.k + k'.	Quantity. 100 grm.	As_2O_3 per 100 grm.	As_2O_3 Total.
1st day...	9. 0 a.m.- 1. 0 p.m.	c.c. 75	gm. 0.109	gm. 0.090	gm. 0.199	45	gm. 0.050	mg. 2.5	mg. 1.9	mg. 0.48	9	gm.	mg.	mg.
	1. 0 p.m.- 4.15 p.m.	75	0.093	0.077	0.170	45	0.053	7.6	5.2	1.60	31			
	4.15 p.m.- 9. 0 a.m.	250	0.500	0.310	0.810	38	0.048	27.0	67.5	4.02	83			
	TOTAL.....	400	0.702	0.477	1.179	40	0.049	(18.7)	74.6	—	—	227	trace	trace
2nd day...	9. 0 a.m.- 1.30 p.m.	50	0.132	0.065	0.197	33	0.044	45.0	22.5	5.00	115			
	1.30 p.m.- 9. 0 a.m.	250	0.445	0.343	0.788	43	0.040	21.4	53.6	2.75	68			
3rd day...	TOTAL.....	300	0.577	0.408	0.985	41	0.041	(25.4)	76.1	—	—	237	3.6	8.5
	9. 0 a.m.- 1.30 p.m.	100	0.146	0.111	0.257	43	0.051	19.6	19.6	4.36	76			
	1.30 p.m.- 4.15 p.m.	60	0.108	0.140	0.248	56	0.091	12.5	7.5	2.73	30			
	4.15 p.m.- 9. 0 a.m.	320	0.459	0.401	0.860	47	0.051	8.0	25.6	1.53	30			
4th day...	TOTAL.....	480	0.713	0.652	1.365	48	0.057	(11.0)	52.7	—	—	180	6.1	11.0
	9. 0 a.m.- 2. 0 p.m.	100	0.155	0.128	0.283	45	0.057	8.1	8.1	1.62	29			
	2. 0 p.m.- 9. 0 a.m.	330	0.434	0.366	0.800	46	0.042	6.8	23.8	1.25	29			
5th day...	TOTAL.....	450	0.589	0.491	1.083	46	0.045	7.1	31.9	—	—	300	1.5	4.5
	9. 0 a.m.- 2. 0 p.m.	100	0.168	0.095	0.263	36	0.053	4.9	4.9	0.98	18			
	2. 0 p.m.- 9. 0 a.m.	200	0.317	0.210	0.527	40	0.038	3.4	6.8	0.36	13			
6th day...	TOTAL.....	300	0.485	0.305	0.790	39	0.033	(3.9)	11.7	—	—	175	0.96	1.7
	9. 0 a.m.- 2. 0 p.m.	275	0.339	0.317	0.656	48	0.027	1.5	4.1	0.17	6.2	306	0.89	2.7
7th day...	TOTAL.....	280	0.356	0.436	0.792	55	0.033	0.93	2.6	0.11	3.3	283	0.42	1.2
	9. 0 a.m.- 2. 0 p.m.	400	0.591	0.811	1.402	58	0.058	0.65	2.6	0.11	1.9	100	0.64	0.64
8th day...	TOTAL.....	225	0.444	0.386	0.830	47	0.035	0.42	0.95	0.04	1.1	—	trace	trace
	9. 0 a.m.- 2. 0 p.m.	200	0.510	0.384	0.894	43	0.037	0.23	0.46	0.02	0.5			
9th day...	TOTAL.....	200	0.510	0.384	0.894	43	0.037	0.23	0.46	0.02	0.5			
	9. 0 a.m.- 2. 0 p.m.	200	0.510	0.384	0.894	43	0.037	0.23	0.46	0.02	0.5			
10th day...	TOTAL.....	200	0.510	0.384	0.894	43	0.037	0.23	0.46	0.02	0.5			
	9. 0 a.m.- 2. 0 p.m.	200	0.510	0.384	0.894	43	0.037	0.23	0.46	0.02	0.5			

Total As_2O_3 recovered in 10 days = 288 mg. = 96 per cent. of the dose..... 257.7

30.3

It will be observed that the general course of elimination is similar to the preceding cases, although with the higher dose a higher percentage recovery is registered: 96 per cent. as against 89 per cent., 85 per cent., and 90 per cent., for the three previous cases on lower doses. Again, about 12 mg. (anything from nil to 26 mg.) remain unaccounted for after ten days. Of the 96 per cent. recovered 86 per cent. (258 mg.) appears in the urine and 10 per cent. (30 mg.) in the faeces. Again the highest output in the faeces (11 mg.) occurs on the third day. The quantities of below 1 mg. (.96 mg.-.64 mg.) excreted in the faeces from the fifth to the tenth day may be residues of unabsorbed arsenic from the original rumenal contents, but it seems equally likely that they correspond to small quantities returned to the intestines by the bile. As in the preceding cases the output in the urine is highest on the first two days, but owing to the larger dose, and partly perhaps to the cachet form of dosing (rumenal path?), the high rate of elimination is continued over the fourth day and the rapid fall does not occur until the evening of the fifth day. On the morning of the fifth day it is still practically 1 mg. per hour. For the first and second day it is 75-76 mg., or 23 per cent. of the dose, for the third day 53 mg., or 13 per cent., for the fourth day 32 mg., or 11 per cent., and for the fifth about 12 mg., or 4 per cent. The highest rate of elimination is reached on the morning of the second day, where it reaches the very high figure of 5 mg. As_2O_3 per hour, but the overnight sample of the first day is not much lower. Within four hours of dosing the arsenic elimination is already appreciable and within eight hours has risen to 1.6 mg. per hour. The hourly rate of elimination in this case does not fall off quite regularly with the time, since the morning sample of the third day is higher than the overnight sample of the second day—4.36 mg. per hour, as against 2.75 mg. This irregularity is probably explainable by an irregular passage of stomach contents on to the intestine, and a consequent irregularity of absorption. That this explanation is the probable one is confirmed by the fact that arsenic injected straight into the bloodstream is eliminated more rapidly and with a more regular rate of diminution as time goes on. This is shown in Tables VIII and IX discussed below in dealing with the fate of arsenic after it reaches the blood. Table VII, however, may be first considered, in which is recorded the elimination of ingested arsenic after double dosing under the precise dietetic conditions recommended in practice.

TABLE VII:

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Table VII.

SHOWING ELIMINATION OF ARSENIC AFTER DOUBLE DOSING FOR WIRE-WORM.

Sheep No. 11010. Good condition; weight 81 lb.

February 25th.—Kept without food and water from 3 p.m., i.e. 17 hours.

February 26th.—Dosed with .625 gm. wire-worm remedy, equivalent to .100 gm. As_2O_3 as sodium arsenite.

February 26th.—2 p.m.—4 p.m. Allowed green food but no water.

4 p.m.—9 a.m. Neither food nor water.

February 27th.—9 a.m. Second dose. Treatment as on previous day.

February 28th.—9 a.m. Food and water *ad lib.* as usual.

Collection periods accurate by observation of time of micturation.

TIME.	COLLECTION PERIOD.	ELIMINATION IN URINE.				IN FAECES.		
		Quantity.	As_2O_3 per 100 c.c.	As_2O_3 Total.	As_2O_3 calc. per Hour.	Quantity.	As_2O_3 per 100 grm.	As_2O_3 Total.
		c.c.	mg.	mg.	mg.	grm.	mg.	mg.
1st day...	9. 0 a.m.—9.40 a.m..	15	1. 7	0. 26	0. 39			
1st dose =	9.40 a.m.—12.20 p.m..	58	10. 3	6. 0	2. 25			
100 mg.	12.20 p.m.—2.55 p.m..	37	28. 1	10. 4	4. 03			
	2.55 p.m.—9. 5 a.m..	290	19. 2	55. 8	3. 68			
	TOTAL.....	400	(18. 1)	72. 5	—	153	nil	nil
2nd day..	9. 5 a.m.—9.50 a.m..	15	14. 0	2. 1	2.80			
2nd dose =	9.50 a.m.—11.55 a.m..	36	14. 2	5. 1	2.45			
100 mg.	11.55 a.m.—4.20 p.m..	74	27. 4	20. 3	4.60			
	4.20 p.m.—9.50 a.m..	270	12. 3	33. 3	1.90			
	TOTAL.....	395	(15. 4)	60. 8	—	65	1. 3	0.88
3rd day..	9.30 a.m.—10.20 a.m..	35	2.02	0.71	1.40			
	10.20 a.m.—12.50 p.m..	152	1.25	1. 9	0.76			
	12.50 p.m.—3.25 p.m..	98	1.52	1. 5	0.58			
	3.25 p.m.—9.40 p.m..	625	1.74	10. 9	0.59			
	TOTAL.....	910	(1.65)	15. 0	—	237	5. 0	11. 8
4th day..	9.40 a.m.—4. 5 p.m..	120	1.66	2. 0	0.31			
	4. 5 p.m.—10.50 a.m..	195	2.52	4. 9	0.29			
	TOTAL.....	325	(2.12)	6. 9	—	220	4. 4	9. 7
5th day..	10.50 a.m.—11.50 a.m..	15	1.05	0.16	0.16			
	11.50 a.m.—9. 0 a.m..	250	0.84	2.10	0.10			
	TOTAL.....	265	(0.87)	2.30	—	127	2. 0	2. 5
6th day..	TOTAL.....	275	0.44	1. 2	0.05	67	1. 8	1. 2
7th day..	TOTAL.....	250	0.72	1. 8	0.07	194	0.82	1. 6.
8th day..	TOTAL.....	350	0.22	0.77	0.03	135	0.61	0.82
9th day..	TOTAL.....	300	0.14	0.42	0.02	—	trace	—
	TOTAL.....	—	—	161. 6	—	—	—	28. 5

Total eliminated in both urine and faeces = 190 mg. = 95 per cent. of that injected.

It will be observed that the general trend of elimination is very similar to that of preceding cases, in which single doses were given; except that owing to the second dosing the high rate of urinary excretion is maintained throughout the second day, while the level of faecal elimination remains high over the fourth day. In the light of

the data for single dosing the figures for double dosing are very much in accord with expectation. It is interesting to note, however, that in this experiment the first day's output of arsenic in the urine is exceptionally high, and nearly as rapid as if the dose had been injected straight into the blood-stream instead of being ingested through the mouth (compare Table VIII). Quite obviously the greater part of the powder must have passed directly to the abomasum and thence rapidly into the intestine for absorption. Over a quarter of a milligram has reached the bladder within forty minutes. Three hours after dosing over 6 per cent. has passed out into the urine, and within six hours 17 per cent. has been dealt with in this fashion. In the ensuing eighteen hours another 56 per cent. is added.

The maximum hourly rate of urinary elimination occurs between the third and sixth hours. It is instructive to compare this sheep with sheep No. 8133 of Table I, with which it was found that 61 per cent. of the ingested dose had disappeared from the stomach system, or 58 per cent. from the whole alimentary tract, within four hours; or with No. 10868 of Table I, where 67 per cent. of the dose had been absorbed within eight hours of administration, and 17 per cent. eliminated in the urine.

Whenever, then, ingested arsenite reaches the intestine quickly it is absorbed with great rapidity and excreted with very little delay. It will presently be shown that the actual rate of elimination after absorption is regulated by temporary abstraction of arsenic from the circulating blood and temporary loose fixation in the tissues. The second dose administered on the second day has also, for the most part, taken the direct abomasal path. Approximately 61 mg. As_2O_3 appears in the urine of the day of dosing, and, since so much of the first dose was eliminated the day before, about 50 mg. of this is suggested as derived from the second 100 mg. dose. A fair proportion, however, is also suggested as having gone to the rumen, since the rate of elimination is not so fast as in the case of the first day. It would appear to correspond in the case of sheep No. 10676 of Table I, in which 58 per cent. of the dose was still present in the stomach system five hours after administration. The drop in the hourly rate of elimination between the 25th and 27th hours after the first dose suggests that the absorption and elimination of the second dose is very small within the first three hours after administration, while the rise to 4.6 mg. per hour in the afternoon (11.55 a.m.-4.20 p.m.) shows that it is well under way within the ensuing few hours. On the third day after the first dose, or second day after the second dose, the elimination falls rapidly; and by the fourth day it is down to 6.9 mg., or 3.5 per cent. of the combined administrations. By the end of the fifth day, the rate has fallen to one-tenth of a milligram per hour, while on the ninth day it is down to one-fiftieth of a milligram per hour, or .42 mg. for the whole day's output. By this time 81 per cent. of the total ingested arsenic has been eliminated in the urine and 14 per cent. in the faeces.

The maximum faecal elimination occurs on the third day after dosing—11.8 mg. on the third day after the first dose, and 9.7 mg. on the third day after the second dose. By the ninth day faecal elimination is ended.

In all, 95 per cent. (± 5) of the arsenic administered has been accounted for. This leaves only 5 per cent. unaccounted for—10 mg., or possibly 20 mg. if the analytical error be credited to the high side

in this instance. There is obviously, then, no marked prolonged storage of arsenic by permanent fixation in the tissues. The 10 mg. concerned only amounts to a trace when distributed over the whole body, and is presumably gradually cleared out in the ensuing few weeks; most probably in the urine, since minute traces can usually be detected there up to three weeks after dosing.

VI.—FATE OF ARSENIC IN THE BLOOD-STREAM.

All the preceding data go to show that, in the sheep at least, the elimination of arsenic in the urine is rapid once it has reached the blood-stream. The statements of various authors in regard to the elimination of arsenic (in general) are very conflicting. Thus, according to Cushny ("Pharmacology and Therapeutics," 5th ed.), "arsenic is excreted very slowly, some appearing in the urine and faeces within twenty-four hours, but only one-fifth of that absorbed being eliminated in this way. The rest is stored in the tissues for a long time and slowly got rid of in the hair and epidermis, in which arsenic may be found for many months after it has disappeared rapidly from the urine and faeces. . . . Arsenic disappears rapidly from the blood when injected, being taken up by the tissues, in which it forms firm combinations with the nucleins."

Witthaus ("Manual of Toxicology"), quoting Severi, states that "when a single dose has been taken the elimination by the urine proceeds rapidly and may cease upon the fourth or fifth day after the administration," but adds that "cases have been reported in which urinary elimination has continued for a much longer period, as long as ninety-three days, even when but a single dose has been taken."

In general it seems that the elimination of arsenic is more protracted in cases of chronic poisoning, or in cases where continuous absorption of small amounts of arsenic has occurred, but the amounts excreted a long time (months) after discontinued absorption are presumably very small and only detectable by very refined methods of a more or less qualitative character.

The difficulty about any of the protocols accessible to us lies in the fact that the conclusions are too frequently based upon qualitative investigation, and that attempts to conduct "balance experiments," in which arsenic excreted is checked against arsenic administered, are few and far between. A demonstration that arsenic disappears from the urine after a given interval does not show that all the arsenic administered has been eliminated, but leaves room for all sorts of conjecture about the amount remaining fixed in the tissues.

Heffter's results on the dog are quoted by Witthaus, in which 38 mg. of arsenic was injected daily (hypodermically as sodium arsenite) but only 26 per cent. (10 mg. average) recovered in the urine, appear to us to be open to serious question.

The results of Almqvist and Welander on the human gave an elimination of 3.3 mg. to 14 mg. As_2O_3 in the urine after daily injection of 20 mg. If our own work on the sheep allows of any analogy with that on the human subject, the higher figure would seem to be much more probable than the lower. It must also be remembered that with repeated small daily injections the opportunity for elimination through the faeces (biliary secretion) is much greater than it is

when a single dose is administered and elimination is too rapid to allow of marked gastro-hepatic circulation.

Our own experiments, in which a balance was always aimed at, have consistently shown that (for the sheep at least) anything up to 72 per cent. (Table VII) of the absorbed arsenic can be eliminated in the first twenty-four hours, and that elimination of ingested arsenic (in urine and faeces) is nearly complete in a week; that the amount then left to be slowly eliminated by urine, faeces, skin, and hair, is a mere fraction of the dose administered, and probably of trifling importance. Unless the human subject, and other animal subjects to which Cushny presumably refers, behave very differently to the sheep, the course of elimination implied in the text-book statement quoted is certainly not representative. It may possibly be representative of cases in which minute amounts are obtained from arsenical waters or beer, but if the amount absorbed is at all high (30 mg., or half a grain, upwards) the idea that only one-fifth of that absorbed is eliminated in the urine does not hold. It is true that "arsenic disappears rapidly from the blood when injected," or rather we should say "the concentration in the blood-stream rapidly falls," but the combinations which it forms in the tissues are certainly not "firm," and very little is permanently held up for any length of time.

Since detailed protocols on any class of subject are so scanty our own protocols for the sheep should be of considerable physiological interest.

The following Table VIII illustrates the rapidity of disappearance of arsenic from the blood-stream after intravenous injection. With sheep No. 13001 the concomitant fixation in the tissues and elimination in the urine is given. Sheep No. 12974 is a duplicate for blood determinations only, samples (100 c.c.) being drawn off at shorter intervals after the injection. Injections were made straight into the jugular vein with a measured syringe, and samples of blood taken off from the same vein at the intervals recorded—working on alternate sides of the neck at each insertion of the needle. Between insertions sheep No. 13001 was confined in a metabolism crate with a urine beaker strapped on. The calculations for total arsenic in the blood circulation are made on the empirical assumption that the blood volume is 8 per cent. of the body weight. They are, therefore, only approximate, but the numbers enable us to form a clear visual impression of what happens to the arsenite injected. The temporary fixation in the tissues is estimated by difference from the total injected and the amount which is still present in the blood or which has been turned out in the urine—interpolated roughly from the hourly rate of elimination when time of micturation does not correspond with time of bleeding. It will be shown presently (Table IX) that the gastro-hepatic circulation of arsenic is small, and faecal elimination can consequently be ignored in this Table VIII.*

* It may be mentioned that C. D. Dijkman, M.A., Demonstrator in Chemistry at Grey College, Bloemfontein, and honorary research worker in this laboratory during the long University vacation, also carried out a similar experiment in the course of investigations on the rate of arsenic elimination in different animals. His results on the sheep were similar to those of Table VIII, and substantially demonstrated the same fate of injected arsenic. His protocols, however, are being utilized elsewhere, and need not therefore be detailed here.

Table VIII.

Sheep No. 13001. Weight 77 lb. = 35 kilos. Injected intrajugularly at 9 a.m. with 50 c.c. aqueous solution of sodium arsenite, equivalent to 100 mg. As_2O_3 . Periods accurate to within a few minutes.

Time after injection. Bleedings.	BLOOD AND TISSUES.			Period of Collection.	URINE.			
	Arsenic stated as As_2O_3 .				Arsenic as As_2O_3 .			
	Per 100 c.c.	Total in circulation.	Tempor- arily fixed in the tissues.		Quan- tity.	per 100 c.c.	Total.	Per hour.
	mg. (3·6)	mg. (100)	mg.		c c.	mg. 18·6	mg. 2·8	mg. 8·4
(Nil; the- oretical)	—	—	—	9. 0 a.m.— 9.20 a.m..	15	—	—	—
20 minutes	0·18	5·0	92	9.20 a.m.—12.40 p.m..	80	11·9	9·5	2·9
2 hours.	0·16	4·5	88	12.40 p.m.— 1.55 p.m..	30	9·0	2·7	2·2
7 hours.	0·10	2·8	78	1.55 p.m.— 9.20 a.m..	175	35·0	61·3	3·2
24 hours	0·01	0·3	23	1st day's total.....	300	(25·4)	76·3	—
—	—	—	—	9.20 a.m.—11.30 a.m..	25	10·4	2·6	1·2
—	—	—	—	11.30 a.m.— 3.30 p.m..	50	10·8	5·4	1·3
—	—	—	—	3.30 p.m.— 8.45 a.m..	520	1·5	7·7	0·45
48 hours	trace	<0·1	8	2nd day's total.....	595	(2·6)	15·7	—
				Total for 48 hours..	—	—	92	—

Sheep No. 12974. Weight 83 lb. = 38 kilos. Injected intrajugularly at 9.0 a.m. with 50 c.c. aqueous solution of sodium arsenite, equivalent to 100 mg. As_2O_3 .

Bledings.	Time after Injection.	Arsenic stated as milligrams As_2O_3 per 100 c.c. of blood.
First.....	10 minutes	·24
Second.....	30 minutes	·21
Third.....	1 hour	·20
Fourth.....	2 hours	·20

It is at once obvious that arsenite injected straight into the blood-stream is immediately abstracted and fixed in the tissues. From an initial "theoretically possible" concentration of 3.6 mg. per 100 c.c. (100 mg. in an estimated total blood volume of 2800 c.c.) there is a very rapid fall to 0.18 mg. per 100 c.c. within twenty minutes. As the second sheep (No. 12974) shows, the fall within ten minutes may be down to 0.24 mg. per 100 c.c., and absorption by the tissues may therefore be regarded as taking place as fast as the arsenic is distributed over the system by the blood. Stated on the total blood volume only 5 mg. (5 per cent.) is circulating in the blood twenty minutes after injection, while 92 per cent. has been fixed in the tissues. Over the next few hours there is a slower fall in blood concentration and a leaching out of tissue arsenic at the rate of 2 to 3 mg. an hour, with concomitant elimination in the urine. By the end of the seventh hour the blood is down to one-tenth of a milligram per 100 c.c., or approximately 3 mg. in the whole circulation, while about 78 per cent. is still fixed in the tissues. At the end of the first twenty-four hours only

traces of arsenic are present in the blood and the 92 per cent. initially fixed in the tissues has been leached down to 23 per cent. After forty-eight hours the total amount of arsenic in the whole circulation is only a fraction of a milligram. The tissues have then parted with most of the rest of their temporarily fixed arsenic, and only 8 mg. can be regarded as still retained. By this time 92 per cent. of the 100 mg. injected has been eliminated in the urine—approximately 76 per cent. on the first day and 16 per cent. on the second. In the urine the *rate* of elimination is high immediately after the injection, 2.8 mg. being turned out in the first twenty minutes, equivalent to over 8 mg. per hour. This appears to be a first flushing effect, since three hours later the rate falls to 2.9 mg. per hour, dropping to 2.2 mg. within the first seven hours. Thereafter there is a slight rise again, suggesting a turn in the tide of the affairs of the tissues—a somewhat increased rate of leaching out of arsenic a few hours after fixation. This secondary rise might, of course, be inconstant, and need have no particular significance, but in Table IX (discussed next), in which the elimination of injected arsenic is followed at more frequent intervals within the first twenty-four hours, the same small rise in hourly rate is observed about seven hours after injection. It only lasts an hour or two (3.10 p.m.-4.35 p.m.), but is quite distinct. The probability is that the secondary rise in Table VIII is sharper than is actually indicated. Unfortunately no evening watch was kept in this case, and only one sample was procured between 2 o'clock in the afternoon and 9 o'clock next morning. Analogy with Table IX suggests that the rate of elimination probably rose in the afternoon to well above the 3.2 mg. per hour actually recorded, and then tailed off overnight to the 1.2 mg. per hour indicated for the morning sample of the second day.

The explanation of the rise is not quite clear, but it may be due to renal intoxication; delayed secretion of urine immediately after the first flushing with arsenic, reducing the hourly elimination for a few hours; subsequent recovery and consequent more rapid elimination. The fall of arsenical concentration in the blood overnight is quite in line with this view, the tissues being regarded as in equilibrium with the blood. On the other hand it might be that, having once fixed the arsenic, the tissues themselves are stimulated to get rid of it again; that there is no back-pressure effect on the part of the kidneys but that renal elimination into the bladder simply follows tissue elimination into the blood. Any way, the phenomenon is not very marked, although if constant it may be of some physiological interest.

On the night of the second day (Table VIII) the rate of elimination falls to .45 mg. per hour, and presumably then tails off in the fashion indicated in Table IX.

Table IX records the elimination of sodium arsenite injected intravenously, followed over a longer period of time and with due consideration to the possibility of faecal excretion of arsenic. Sheep No. 11010, already used in several of the other experiments detailed here, was again used for this test. The amount of arsenic injected was smaller than in the case of Table VIII, the arsenite being dissolved in saline and 55 c.c., equivalent to 57 mg., As_2O_3 , being used.

At this point it may be mentioned that it is fairly safe to inject anything up to 150 mg. As_2O_3 as sodium arsenite into a sheep weighing 35-50 kilos. We have injected 120 mg. on three occasions, 150 mg. in one case, and 200 mg. in another, without serious consequences. On another occasion, however, 200 mg. proved rapidly fatal. We are inclined to think that about 150-200 mg. represents the average toxic dose by intravenous injection.

In considering Table IX (as also most of the other tables) attention may be drawn to the fact that division into "days" is only approximate, e.g. the second day is entered from 8.30 a.m. to 10.10 a.m. the following morning. This is due to the method of recording periods according to the time of micturation. If 24-hour data are wanted, a closer approximation can be obtained by interpolation from the data actually given.

TABLE IX:

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Table IX.

Sheep No. 11010. Weight 81 lb. Intravenous injection (jugular) of sodium arsenite equivalent to 57 mg. As_2O_3 at 8.35 a.m. Sodium arsenite solution in saline made up approximately 0.1 per cent. As_2O_3 and checked by titration; delivery of syringe tested and found to be 55 c.c. Sheep kept under constant observation so that periods of micturition correspond to the time stated to an accuracy of 5 minutes. Quantities of urine are stated accurately within 5 c.c., measurements being taken before rinsing out the collecting beaker with water and making up to volume taken for analysis. Creatinine, creatine, and As_2O_3 figures therefore correspond accurately to the periods recorded. First day's urine up till 8.30 in the evening, analysed for every micturition; subsequent days combined as indicated. Creatinine + creatine by Benedict's modification of original Folin method. No preliminary dietic treatment.

TIME.	COLLECTION PERIOD.	ELIMINATION OF URINE										IN FÆÆ.	
		Creatinine k.	Creatine k'.	k + k'.	k' as Per- centage of k + k'.	k + k' per Hour.	As_2O_3 per 100 c.c.	As_2O_3 Total.	As_2O_3 calculated per Hour.	Mg. As_2O_3 per Grm.k + k'.	Quan- tity.	As_2O_3 per 100 grm.	As_2O_3 Total. mg.
1st day....	8.35 a.m.-9.5 a.m.	grm. 0.031	grm. nil	grm. 0.031	nil	grm. —	mg. 5.86	mg. 2.05	mg. >4.10	—	—	mg.	—
	9.5 a.m.-10.15 a.m.	0.046	nil	0.046	nil	0.039	7.0	3.50	3.00	76	—	mg.	—
	10.15 a.m.-1.10 p.m.	0.087	0.005	0.092	5	0.032	8.0	6.00	2.06	65	—	mg.	—
	1.10 p.m.-3.10 p.m.	0.056	0.007	0.063	11	0.032	12.0	4.20	2.10	67	—	mg.	—
	3.10 p.m.-4.35 p.m.	0.043	0.019	0.062	31	0.044	12.3	3.70	2.61	60	—	mg.	—
2nd day...	4.35 p.m.-8.30 p.m.	0.120	0.052	0.172	31	0.044	19.1	8.08	2.06	47	—	mg.	—
	8.30 p.m.-8.30 a.m.	0.344	0.087	0.431	20	0.036	4.08	10.2	0.85	24	—	mg.	—
	TOTAL.....	0.727	0.170	0.897	19	0.037	(6.8)	37.7	—	—	430	nil	nil
	8.30 a.m.-11.0 a.m.	0.080	0.027	0.107	25	0.043	3.09	1.70	0.68	16	—	mg.	—
	11.0 a.m.-3.45 p.m.	0.144	0.061	0.205	30	0.043	1.65	1.65	0.35	8	—	mg.	—
3rd day...	3.45 p.m.-10.10 a.m.	0.465	0.335	0.800	42	0.044	1.24	4.05	0.22	5	—	mg.	—
	TOTAL.....	0.689	0.423	1.112	3*	0.043	(1.54)	7.4	—	—	554	0.16	0.91
	10.10 a.m.-3.10 p.m.	0.67	0.214	0.881	56	0.076	0.42	0.71	0.14	1.9	—	mg.	—
	3.10 p.m.-10.40 a.m.	0.463	0.434	0.897	48	0.046	0.72	2.15	0.11	2.5	—	mg.	—
	TOTAL.....	0.630	0.648	1.278	51	0.052	(0.61)	2.86	(0.12)	—	362	0.14	0.52
4th day...	10.40 a.m.-10.10 a.m.	0.365	0.705	1.070	66	0.045	0.42	1.70	0.07	1.6	—	mg.	—
	10.10 a.m.-10.20 a.m.	0.611	0.799	1.410	56	0.058	0.23	0.88	0.04	0.6	—	mg.	—
	10.20 a.m.-11.20 a.m.	0.648	0.324	0.972	33	0.039	0.22	0.60	0.02	0.6	—	mg.	—
	11.20 a.m.-9.30 a.m.	0.510	0.196	0.706	28	0.032	0.17	0.55	0.02	0.8	—	mg.	—
	TOTAL.....	—	—	—	—	—	—	51.7	—	—	—	mg.	1.74

Total As_2O_3 recovered in seven days = 53.4 mg. = 94 ± 5 per cent. of the injected dose.

The general trend of elimination is very similar to that shown in Table VIII, and differences are only such as might be expected in consideration of the fact that the amount of arsenic injected was smaller. Thus 37.7 mg. out of 57 mg., or 66 per cent., is eliminated within the first twenty-four hours as against 76.3 mg. out of 100 mg., or approximately 76 per cent., in the previous case. On the second day the elimination is 13 per cent. of the dose, as against approximately 16 per cent. for the preceding experiment; a total of 79 per cent. in forty-eight hours as against 92 per cent. Thus, although elimination in both cases is rapid, the lower dose is indicated as coming out rather more slowly than the higher.

On the third day the urinary elimination has dropped to 5 per cent. of the dose, or a little under 3 mg. absolute excretion. Thereafter the output tails off at 1.7 mg., .88 mg., .6 mg., .55 mg., or 3 per cent., 1.5 per cent., 1.1 per cent., 0.97 per cent., on the 4th, 5th, 6th, and 7th days respectively. By this time 91 per cent. of the amount injected has appeared in the urine and about 3 per cent. in the faeces. This only leaves 6 per cent., or about $3\frac{1}{2}$ mg. (possibly up to 7 mg.) for subsequent protracted elimination.

Faecal elimination, as might be expected, is small, but it is interesting to note that it is appreciable. Although the arsenite was injected straight into the blood-stream, an easily detected fraction appears in the faeces—a total of 1.7 mg. from the second to the fourth day inclusive. This small absolute amount might possibly be accounted for by endosmosis through the intestinal walls, but it is more probably attributable to biliary excretion. Arsenic may be detected in the bile of dosed sheep and cattle, although in the few tests which we have had an opportunity of making the amount was very small. Since collection of bile at intervals after dosing is practically out of the question, and since the rate of bile flow is not known, a quantitative estimate of the extent of biliary secretion of arsenic is very difficult to arrive at. Nevertheless, the mere presence of arsenic at all indicates at least a limited circulation between liver and intestines. Lander (*Veterinary Toxicology*, p. 38) doubts this gastro-hepatic circulation of arsenic, but our own experiments suggest that a limited cycle of biliary excretion, reabsorption from the intestine and return to the liver, does take place, although the proportion of any given dose which enters into such circulation is probably very small. In the case of Table IX it seems rational to regard the 1.7 mg. found in the faeces as the unabsorbed balance of the arsenic secreted in the bile, and to conclude that the total amount involved in the gastro-hepatic circulation was somewhat higher; 5 mg. perhaps, or anything up to 10 per cent. of the amount injected. The actual amount involved would, of course, vary with the dose and method of dosing, but since elimination through the urine is so rapid it could never be very high.

In the case of very small administrations, of, say, a few milligrams, the absolute amount excreted in the faeces would, of course, be very low, but, since the rate of urinary elimination would probably be slower owing to easier retention by the tissues, more time would be available for gastro-hepatic circulation, and an apparently higher percentage of the small dose might be expected in the faeces.

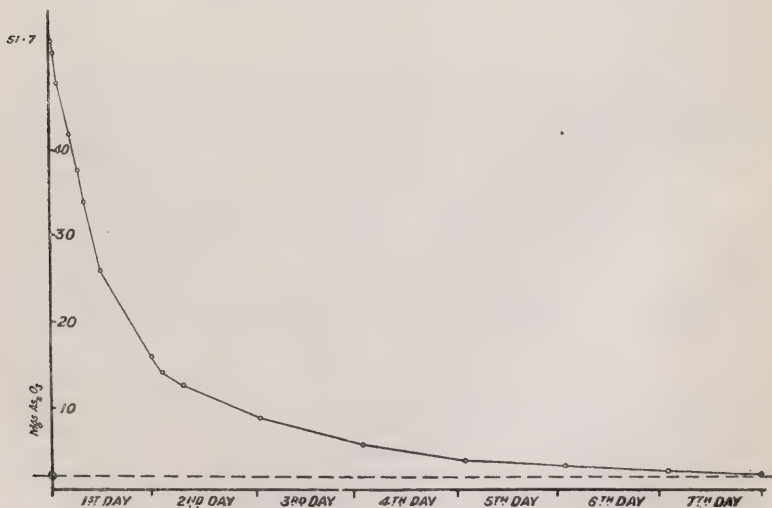
In regard to the rate of urinary elimination it may be noted that the output per hour in Table IX is analogous to that recorded in Table

VIII, but owing to the more frequent collection of urine it is shown in more detail. The first sample passed half an hour after injection contains 2.05 mg. As_2O_3 —a rate of 4.1 mg. per hour. This “flushing” rate falls to 3 mg. within the next eighty minutes, and to 2 mg. within the next three hours. About the seventh hour (3.10 p.m.-4.15 p.m.) a small secondary rise, already commented upon, occurs, but within the following six hours the rate is back to 2 mg. On the whole, however, we may regard the rate as high and fairly steady during the first twelve hours after injection. By this time 27.5 mg., or nearly half the dose, has been turned out by the kidneys. Within the second twelve hours the rate drops to .85-.68 mg. per hour, or to about one-third that of the first twelve hours. It may be recalled that in Table VIII, where the arsenite injected was greater, a higher rate of elimination was maintained throughout the early part of the second day—1.3 mg. per hour twenty-six to thirty hours after injection.

On the second day the rate falls rapidly, dropping to .35 mg. per hour in the afternoon and .22 mg. overnight. Thereafter it drops from .12 mg. on the third day down to one-fiftieth of a milligram per hour at the end of the week.

The accompanying curve gives a graphic representation of the rate of elimination recorded in Table IX.

The highest ordinate, marked 51.7, corresponds to the 51.7 mg. As_2O_3 eliminated in the urine out of the 57 mg. injected. The curve then shows the rate at which the arsenic disappears from the system by elimination in the urine.



Distribution of Arsenic in the Tissues after Immediate Abstraction from the Blood.—It was considered of interest to follow the arsenic from the blood-stream into the tissues in order to complete the picture and effect a clear visualization of the fate of injected arsenite. Table X gives analyses of the tissues of a sheep injected intrajugularly with

arsenite equivalent to 100 mg. As_2O_3 (as in Table VIII), and killed *thirty minutes* after injection, i.e. after the first preliminary equilibrium had been reached. The tissues are arranged in order of descending arsenical concentration expressed as mg. As_2O_3 per 100 gm.

Table X.

Sheep No. 12995. Weight 81 lb.=37 kilos. Injected intrajugularly at 9 a.m. with 55 c.c. aqueous solution of sodium arsenite equivalent to 100 mg. As_2O_3 . Killed at 9.30 a.m. for analysis.

(\pm indicates nil or a possible trace).

Tissue.	Weight in gm.	As_2O_3 per 100 gm.	Total As_2O_3 .
		mg.	mg.
Liver.....	580	2.05	11.9
Kidneys.....	95	1.95	1.85
Lungs.....	735	0.58	4.3
Heart.....	173	0.49	0.85
Muscles (gluteus).....	—	0.31	(about 57 ?)
Blood.....	(2,960)	0.21	(6.2)
Spleen.....	73	0.18	0.13
Suprenals.....	9	\pm	—
Brain.....	107	\pm	—
Bones (femur).....	—	\pm	—
Bile.....	Gall bladder empty	—	—
Intestinal Contents.....	570	\pm	—
Urine in Bladder.....	8	25	2.0

It is to be noted that the liver and kidneys, with approximately 2 mg. As_2O_3 per 100 gm. of tissue, fix most arsenic per unit mass, although they only account for a small proportion of the absolute temporary fixation; the liver taking approximately 12 per cent. and the kidneys approximately 2 per cent. of the total administration.

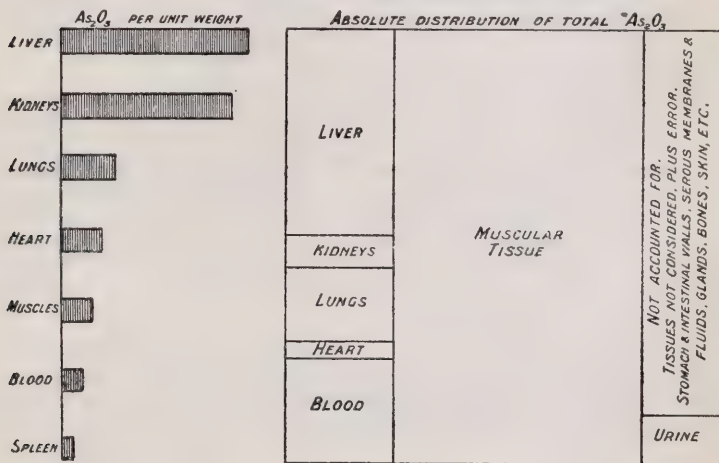
Lungs come next with .58 mg. per 100 gm., totalling rather over 4 per cent. of the dose. Heart muscle and limb muscle fix less than a quarter of the liver fixation per unit weight, heart (.49 mg.) running appreciably higher than gluteus (.31 mg.). The blood itself comes next with .21 mg. per 100 gm., or approximately 6 mg. in all if the total blood volume be accepted as equivalent to about 8 per cent. of the body weight. The spleen is still lower, while the suprenals show a mere trace. The brain had not yet had time to fix any readily detectable amount of arsenic, nor, as might be expected from the brevity of the period after injection (thirty minutes), had the bones. The gall bladder was practically empty, so that the figure for bile is missing, but there is little doubt that had any bile been secreted by the liver after injection it would have contained at least traces of arsenic. The intestinal contents had not yet received the traces of arsenic which, as faecal elimination in Table IX shows, they do ultimately receive. Elimination through the kidneys had already begun at the rate of 4 mg. per hour, and, although only 8 c.c. of urine was found in the bladder at death, 2 mg. As_2O_3 was present.

Taking liver, kidneys, lungs, heart, spleen, and blood together, we can account for just over one-quarter of the total arsenic injected.

Deducting the 2 mg. in the urine we are left with 73 mg. to account for. The greater part of this is obviously fixed in the bulky muscular tissues. If we assume uniform distribution over all the muscles at the level recorded for the gluteus, and assess the total muscular tissue as about half the weight of sheep, we could account for 57 mg. As_2O_3 so fixed. This would only leave us with 16 mg. not accounted for. It was, of course, not the intention to attempt to account for all the arsenic injected but only to obtain an approximate idea of its general distribution.

This general distribution may be better visualized by means of a diagram:—

Showing distribution of sodium arsenite = 100 mg. As_2O_3 , in the tissues of a sheep half an hour after intravenous injection.



Recapitulation.—The general picture of the fate of arsenic after absorption (in the sheep) is therefore fairly clear, and may be recapitulated thus:—

1. Removed from the blood by the tissues as fast as it enters, the circulating blood only retaining a few per cent. of the amount absorbed.

2. Fixed in the tissues to the extent of 90 per cent. or more, but only in loose, temporary fashion, the liver and kidneys fixing most per unit weight of tissue, but the muscles fixing most when the absolute amount of arsenic is considered. When the arsenic enters the blood by absorption from the intestines, the liver may “sieve” the portal blood, and a much higher proportion may be fixed in hepatic tissue than is fixed after injection.

3. Rapidly leached out from the tissues and eliminated in the urine. As much as 15 per cent. of that absorbed may appear in the urine within the first five or six hours, and as much as 75 per cent. within the first twenty-four hours. Of the remainder the greater part comes out on the second day, and considerably less on the third day.

Thereafter the elimination tails off gradually, a fractional percentage being still recoverable after seven days, and a barely detectable trace being present a fortnight later.

4. A small proportion may enter the gastro-hepatic circulation, and a few per cent. of the amount absorbed into the blood may be thus eliminated in the faeces.

5. Other channels of elimination, such as perspiration, wool, etc., are negligibly small.

6. The quantitative behaviour of absorbed arsenic depends upon the magnitude and path of the administration, but with large single sub-toxic doses (50 to 150 mg. for the sheep) 80 to 90 per cent. may be eliminated in the first two to three days, and up to 95 per cent. within a week. The amount firmly fixed in the tissues and undergoing long protracted elimination is therefore very small.

VI.—ABSORPTION AND ELIMINATION OF SODIUM ARSENATE AND OF ARSENIOS OXIDE.

Arsenate.—It was considered of interest to include at least one experiment to determine whether there was any difference between the absorption and elimination of arsenite and of the less toxic arsenate. On theoretical grounds one could not readily predict a difference, although one might possibly expect that in the course of food digestion arsenate would be more likely to pass into insoluble form and so appear in larger amount in the faeces.

In the experiment recorded in Table XI, however, it is evident that the elimination of ingested arsenate follows much the same course as that of arsenite in Table IV, where the same sheep was used and dosing carried out in similar fashion:—

TABLE XI:

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Table XI.

Sheep No. 11010. Drenched 9 a.m. with 100 c.c. water containing sodium arsenate, As_2O_3 equivalent to 100 mg. As_2O_3 . Data expressed as As_2O_3 . Urine quantities made up to figures indicated with a few c.c. of rinsing water. Periods only approximate

ELIMINATION IN URINE.															IN FAECES.		
TIME.	COLLECTION PERIOD.	Quant- ity.	Creatinine k.	Creatine k'.	k + k'.	k' as Per- centage of k + k'.	k + k' per Hour.	As ₂ O ₃ per 100 c.c.	As ₂ O ₃ Total.	As ₂ O ₃ calculated per Hour.	Mg. As ₂ O ₃ per gram. k + k'.	Quant- ity.	As ₂ O ₃ per 100 grm.	As ₂ O ₃ Total.			
															mg.	mg.	mg.
1st day....	9. 0 a.m.- 4. 0 p.m.	c.c. 80	gm. 0.160	gm. 0.113	gm. 0.273	41	0.039	13.5	16.9	2.4	82						
	4. 0 p.m.- 9. 0 a.m.	250	0.486	0.189	0.675	28	0.040	12.2	30.5	1.8	45						
	TOTAL.....	330	0.646	0.302	0.948	32	0.039	(14.4)	47.4	—	—	189	0.45	0.95			
2nd day...	9. 0 a.m.- 4.30 p.m.	150	0.201	0.123	0.324	38	0.043	5.0	7.5	1.0	23						
	4.30 p.m.- 9. 0 a.m.	400	0.475	0.215	0.690	31	0.042	2.4	9.6	0.52	14						
	TOTAL.....	550	0.676	0.338	1.014	33	0.042	(3.0)	16.5	—	—	314	1.4	4.4			
3rd day...	9. 0 a.m.- 4. 0 p.m.	110	0.197	0.054	0.251	21	0.036	1.6	1.8	0.26	7						
	4. 0 p.m.- 9. 0 a.m.	250	0.403	0.147	0.550	27	0.032	1.4	3.5	0.21	6						
	TOTAL.....	360	0.600	0.201	0.801	25	0.033	(1.5)	5.3	—	—	218	1.1	2.4			
4th day...	9. 0 a.m.- 3. 0 p.m.	110	0.097	0.049	0.146	33	0.024	0.83	0.91	0.15	6						
	3. 0 p.m.- 9. 0 a.m.	220	0.290	0.213	0.503	42	0.028	0.91	2.0	0.11	4						
	TOTAL.....	330	0.387	0.262	0.649	40	0.027	(0.88)	2.9	—	—	197	0.48	0.95			
5th day...	9. 0 a.m.- 4.30 p.m.	350	0.113	0.134	0.247	54	0.033	0.34	1.2	0.16	5						
	4.30 p.m.- 9. 0 a.m.	320	0.305	0.081	0.386	23	0.023	0.48	1.5	0.09	4						
	TOTAL.....	670	0.418	0.215	0.633	34	0.026	(0.40)	2.7	—	—	295	0.34	1.0			
6th day...	TOTAL.....	350	0.437	0.187	0.624	30	0.026	0.55	1.9	0.08	3	252	trace	trace			
	TOTAL.....	400	0.315	0.232	0.547	42	0.023	0.17	0.68	0.03	1						
	TOTAL.....	320	—	—	—	—	—	0.12	0.38	0.02							
Total recovered in 8 days expressed as As ₂ O ₃ = 88 mg. = 88 ± 5 per cent. of the dose.....															78.4	9.6	

It will be observed that 88 per cent. (possibly 93 per cent.) of the ingested arsenate is recovered in eight days, 78.4 mg. being eliminated in the urine and 9.6 mg. in the faeces. In the parallel experiment with arsenite (Table IV) the recovery was practically the same—85 per cent. (possibly 90 per cent.), of which 76.5 mg. appeared in the urine and 8.1 mg. in the faeces. In Table III the recovery after drenching with arsenite was 89 per cent., of which 74.9 mg. is recorded for urine and 14.2 mg. for faeces.

As with arsenite, the greater part of the arsenate is eliminated in the urine in the first forty-eight hours after dosing—about 47 per cent. on the first day and 17 per cent. on the second day. Thereafter the elimination tails off at 5.3, 2.9, 2.7, 1.9, .68, and .38 per cent. on the succeeding six days. Such minor differences as are noticeable in comparing the data concerned are arbitrary, and no real distinction can be made out between arsenite and arsenate in regard either to absorption or elimination. It may be mentioned in passing that arsenate, which is less toxic than arsenite for sheep, is also less toxic for *Haemonchus contortus*. It is, therefore, not so serviceable as a constituent of a wire-worm remedy.

Absorption and Elimination of Arsenious Oxide.—It is generally recognized that arsenious oxide, when administered by the mouth, is much less poisonous than an equivalent amount of sodium arsenite, and observations at this laboratory have indicated that this is also markedly true for sheep. The explanation generally offered is that solution of the solid oxide in the alimentary tract is very imperfect, and that, as a consequence, the greater part of the dose escapes absorption. It would appear from the data of other workers on other animals, and the data adduced below (Table XII) for the sheep, that this explanation is the correct one, although on general theoretical grounds it is not immediately obvious why a small amount of arsenious oxide taken in by the mouth should escape solution in the large amount of fluid in the tract. The solubility of arsenic trioxide powder in water at ordinary temperature is quoted by Seidell as about 2 per cent. for the crystalline modification, and 3.7 per cent. for the amorphous modification. The dose of 0.1 gm. given is, therefore, capable of solution in a few c.c. of water, and it seems odd that it should escape solution on its slow journey through the rumen, abomasum, and intestinal tract. On the contrary, one would expect moderate amounts of arsenious oxide to be converted into sodium arsenite in the duodenum, and therefore to behave like arsenite in regard to absorption, elimination, and toxic effect. Nevertheless, this is not the case. Arsenious oxide administered in powder form is largely eliminated in the faeces, whereas, if administered *in solution in water*, it behaves like sodium arsenite, and is eliminated chiefly in the urine. Table XII gives the data obtained on sheep 11010 dosed with 100 mg. of solid arsenious oxide in cachet form, and Table XIII the data on sheep 9545 dosed with the same quantity of arsenious oxide dissolved up in 100 c.c. of water.

Table XII.

Sheep 11010. Dosed with 100 mg. As_2O_3 (ordinary chemically pure arsenious oxide) in cachet form 9 a.m. Quantities of urine made up to the figures given, with a little water used to rinse out the collecting vessel.

COLLECTION PERIOD.	ELIMINATION IN URINE.			IN FAECES.		
	Quantity.	As_2O_3 per 100 c.c.	As_2O_3 Total.	Quantity.	As_2O_3 per 100 grm.	As_2O_3 Total.
	c.c.	mg.	mg.	grm.	mg.	mg.
First day.....	210	0·26	0·55	403	nil	nil
Second Day.....	400	0·23	0·92	290	0·62	1·8
Third day.....	1,000	0·44	4·4	362	5·2	18·8
Fourth day.....	500	0·54	2·7	302	11·4	34·4
Fifth day.....	570	0·37	2·1	321	4·7	15·1
Sixth day.....	450	0·24	1·1	254	1·3	3·3
Seventh day.....	400	0·23	0·92	322	0·48	1·5
Eighth day.....	600	0·12	0·72	484	0·15	0·73
Ninth day.....	350	0·06	0·21	358	trace	trace
TOTAL.....	—	—	13·6	—	—	75·6

Total recovered in nine days = 89·2 mg. = 89 ± 5 per cent. of the dose.

TABLE XIII:

ENSUING PAGE 528.

Table XIII.

Sheep No. 9545. Drenched with 100 c.c. of an aqueous solution of arsenious oxide containing 100 mg. As_2O_3 , 9 a.m., 6th November, 1917. Quantities of urine approximately accurate, but made up to round numbers with a few c.c. of water used for rinsing out the collecting beaker.

TIME.	COLLECTION PERIOD.	ELIMINATION IN URINE.										IN FAECES.		
		Qua- n- ti- ty.	Creatinine k.	Creatine. k.	k + k'.	k' as Per- centage of k + k'.	k + k' per Hour.	As_2O_3 per 100 c.c.	As_2O_3 Total.	As_2O_3 calculated per Hour.	Mg. As_2O_3 per Grm.k + k'.	Quant- ity.	As_2O_3 per 100 grm.	As_2O_3 Total.
1st day...	9. 0 a.m.- 4. 0 p.m.	c.c. 80	gm. 0.163	gm. 0.122	gm. 0.285	43	gm. 0.040	mg. 16. 0	mg. 12. 8	mg. 1.83	45	gm.	mg.	mg.
	4. 0 p.m.- 9. 0 a.m.	450	0.516	0.588	1.101	53	0.065	7. 5	33. 7	1.98	36			
	TOTAL.....	530	0.679	0.710	1.386	51	(0.058)	(8. 8)	46. 5	—	—	532	0.87	4. 6
2nd day...	TOTAL.....	360	0.534	0.436	0.970	45	0.040	3. 8	13. 7	0.57	14	2 0	2. 4	6. 2
3rd day...	TOTAL.....	440	0.674	0.494	1.168	42	0.049	1. 6	7. 0	0.29	6	252	1. 8	4. 5
4th day...	TOTAL.....	310	0.483	0.312	0.795	39	0.033	0.64	2. 0	0.08	2.5	163	0.92	1. 6
5th day...	TOTAL.....	250	0.432	0.291	0.723	40	0.030	0.43	1. 1	0.05	1.5	245	0.69	1. 7
6th day...	TOTAL.....	350	0.500	0.400	0.900	44	0.037	0.21	0.74	0.03	0.8	193	0.39	0.75
7th day...	TOTAL.....	350	0.528	0.388	0.916	42	0.038	0.12	0.42	0.02	0.4	155	0.45	0.70
8th day...	TOTAL.....	300	0.433	0.322	0.755	43	0.032	0.07	0.21	0.01	0.3	138	0.36	0.50
Total As_2O_3 recovered = 92.2 mg. = 92 ± 5 per cent. of the dose.....														71. 7
														20. 5

Experiments with sodium arsenite had already been carried out with both of these sheep, and with both the arsenic had been eliminated mainly in the urine. The data of Tables XII and XIII are, therefore, comparable with the data for arsenite elimination. A duplicate experiment with arsenious oxide was also carried out on sheep No. 11010; 100 mg. worked into a ball of rice dough instead of being enclosed in a cachet. Unfortunately, the animal did not swallow at once, but chewed up the ball and ejected a part of the dose, so that the total recovery of arsenic was only 65 per cent. instead of the usual 85 to 90 per cent. Of the 65 mg. recovered, however, 51 mg. appeared in the faeces and only 14 mg. in the urine. Hence, although the protocols are not worthy of detailed record, they amply confirm the main fact brought out in Table XII, i.e. that with solid arsenious oxide the greater proportion of the dose is eliminated in the faeces and comparatively little in the urine.

For the sake of simplicity in comparing the figures, Tables III, XII, and XIII may be condensed in the form of arsenic elimination totals for eight days after dosing.

Table XIV.

Comparing the elimination of arsenious oxide administered in powder form, in solution in water, and in the form of sodium arsenite.

Table.	III.		XII.		XIII.	
Dose.	100 mg. As_2O_3 as sodium arsenite in 100 c.c. water.		100 mg. As_2O_3 as such in powder form in cachet.		100 mg. As_2O_3 as such dissolved in 100 c.c. water.	
Elimination.	In Urine. Mg. As_2O_3 .	In Faeces. Mg. As_2O_3 .	In Urine. Mg. As_2O_3 .	In Faeces. Mg. As_2O_3 .	In Urine. Mg. As_2O_3 .	In Faeces. Mg. As_2O_3 .
1st day...	24.9	1.3	0.55	1.8	46.5	4.6
2nd day...	23.9	1.8	0.92	18.8	13.7	6.2
3rd day...	13.5	4.4	4.4	34.4	7.0	4.5
4th day...	4.3	3.9	2.7	15.1	2.0	1.6
5th day...	2.4	2.0	2.1	3.3	1.1	1.7
6th day...	2.0	0.6	1.1	1.5	0.74	0.75
7th day...	2.4	0.2	0.92	0.73	0.42	0.70
8th day...	0.7	trace	0.72	trace	0.21	0.50
Total recovered in 8 days	74.1	14.2	13.4	75.6	71.7	20.5
	88.3		89.0		92.2	

It will be observed that in all three cases approximately the same proportion (about 90 per cent.) of the dose is recovered; that the course of elimination of arsenious oxide in solution is very similar to that of sodium arsenite, the ratio of arsenic in the urine to arsenic in the faeces being 74:14 in one case and 72:20 in the other; but that the course of elimination of arsenious oxide administered in powder form is reversed, the ratio now being 13:75 instead of 74:14.

It does not appear to matter whether arsenious oxide be given as such or as sodium arsenite, provided it be in solution, and, as comparison with Tables V and VI shows, it is more or less immaterial whether the readily soluble arsenite be administered in solution or in loose powder form as in dosing for wire-worm, or in cachet form. But the data leave little room to doubt that arsenious oxide administered in powder form (cachet or dough) may actually pass through the alimentary system largely undissolved and appear in the faeces. As already remarked, such behaviour is somewhat odd when the degree of solubility of arsenious oxide in water, and still readier solubility in alkaline media (first intestine), is taken into consideration.

It is obviously the *rate* of solution and not the extent of solubility which is the governing factor in absorption. The powder used was Merck's G.R., and on testing it was found that, although on prolonged shaking of large excess with limited water the text-book solubility could be reached, the solution of limited amounts in large excess of water was very gradual. Though fine and even in texture the powder consisted (microscopically) of crystal fragments of widely varying size, and since the smaller particles dissolved more rapidly than the larger the quantity dissolved in any given time depended rather on the amount of powder taken than on the volume of water used. Thus, on continuous shaking of 0.1 grm. quantities with 10 c.c., 50 c.c., and 100 c.c. of water for six hours, only about 0.01 grm. was dissolved in each case; while on shaking 0.5 grm. with the same quantities of water 0.06 to 0.1 grm. passed into solution. 0.1 grm., the dose used in Table XII, could be left in a litre of water at 37° C., with intermittent shaking, for twenty-four hours and still show the major portion undissolved. Table XIV (or XII) shows that about 75 mg. was passed out in the faeces, and the solubility of Merck's G.R. arsenious oxide under the conditions of food digestion in the sheep therefore amounts to about 25 per cent. for ingested amounts of 100 mg. With coarser samples the absorption would certainly be still less complete.

It may be recalled that Cloetta (*vide* Witthaus), working on the dog, concluded that with repeated administration of arsenious oxide the extent of absorption by the intestine diminished. A dog of 7 kilo weight, which had become habituated to the enormous dose of 3 grm. *daily*, was injected with .04 grm. in solution hypodermically and promptly died. There is little doubt that in arsenophagia in man many of the cases of high tolerance for arsenic are to be attributed to very low absorption of coarse arsenious oxide from the intestine. There is equally little doubt that nothing like so high a tolerance could ever be established for the easily soluble arsenites.

VII.—CREATININE AND CREATINE OUTPUT.

The figures included in the tables used need not come under very detailed discussion in this paper, but, since they were only determined by the wayside, may for the moment merely be left on record. As mentioned earlier, we hope to extend the observations to cover output under varying dietetic conditions and make a separate short communication on the normal creatine-creatinine elimination of the

sheep. In Table IV the daily output of $k+k'$ varies from .672 grm. to .911 grm. for a sheep of 81 lb. live-weight in good condition. This may be stated as $.79 \pm .12$ grm., or $.021 \pm .003$ grm. per kilo.; a variation of about 15 per cent. either way. If we consider that the preliminary starvation period and subsequent dosing with arsenic influenced the output of the first three days, but treat the last six days as normal, we have a smaller variation: from .672 grm. to .725 grm., i.e. $.698 \pm .027$ grm., or about 4 per cent. either way.

But in Table XI the same sheep, with no preliminary treatment, varies still more widely: from .547 to 1.014. Again the highest figure occurs after dosing with arsenic, and may have been influenced by metabolic disturbance, although there is no clear evidence for such an assumption. In Table V, with a different sheep the variation over six days is from 1.161 grm. to .626 grm., i.e. $.893 \pm .268$, or $.024 \pm .007$ grm. per kilo.; a variation of ± 30 per cent. or 60 per cent. between extremes. This time the highest figure is for the fourth day, by which time arsenic elimination is nearly over, so that it is less likely to be due to metabolic disturbance. Excluding this day's output, however, the daily elimination would show up as more regular: $.726 \pm .10$ grm. or about $.024 \pm .007$ grm. per kilo. For sheep No. 9545, Table XIII, the variation is from 1.386 grm. to .723 grm., and even if we exclude the first few days, we still have a variation from .916 to .723 or about $.023 \pm .003$ per kilo.

When the elimination over shorter periods (a few hours) is considered the variation may be greater still. For sheep No. 9724, Table V, omitting the first few days, the rate varies from .065 grm. per hour on the morning of the fourth day to .026 per hour for the sixth day, i.e. $.046 \pm .02$ grm.

The ratio of creatine to creatinine, or the proportion of creatine expressed as percentage of total "creatinine plus creatine" is also very variable. In Table IV the variation is from 17 per cent. to 50 per cent., although in the main the figures run at about 45 per cent. For sheep No. 11010, Table IX, the results are more striking, since the first two samples contain practically no creatine at all, while on the fourth day the proportion is up to 66 per cent. of the total. At first we were inclined to consider that the administration of arsenic (and in some cases the preliminary dietetic treatment) had upset the metabolism and increased the relative proportion of creatine to creatinine; also perhaps that the variations in the daily and hourly rate of $k+k'$ elimination were in part attributable to the same disturbance. A general survey of all the data, however, can not be said to show any constant effect attributable to arsenic, and subsequent data on undosed sheep have shown variations nearly as erratic as those recorded here. The following control figures (Table XV) representing a three-day run on the sheep (No. 11010) used for Table IX, taken a few weeks after cessation of experiment when the urine was arsenic-free and the animal quite normal, are interesting for comparison.

Table XV.

Three days creatinine and creatine output in sheep No. 11,010, under control conditions without arsenical treatment. Body weight 81 lb. = 37 kilos. *Diet*:—The usual ration of hay, lucerne, and maize.

Time.	Collection Period.	Crea- tine k.	Crea- tinine k'.	k + k'.	k' as percen- tage of k + k.	k per Hour.	k' per Hour.	k + k' per Hour.
		gram.	gram.	gram.		gram.	gram.	gram.
1st day...	8.35 a.m.—11. 5 a.m..	0.069	0.007	0.076	9. 2	0.028	0.003	0.031
	11. 5 a.m.—1.25 p.m..	0.061	0.009	0.070	12. 8	0.026	0.004	0.030
	1.25 p.m.—2.50 p.m..	0.055	0.010	0.065	15. 4	0.039	0.007	0.046
	2.50 p.m.—3.45 p.m..	0.036	0.008	0.044	18. 2	0.039	0.009	0.048
	3.45 p.m.—9.45 a.m..	0.514	0.226	0.740	30 5	0.029	0.012	0.041
	TOTAL.....	0.735	0.260	0.995	26. 1	0.030	0.010	0.040
2nd day..	9.45 a.m.—10.35 a.m..	0.030	0.015	0.045	33. 3	0.036	0.018	0.054
	10.35 a.m.—12.55 p.m..	0.073	0.051	0.124	41. 1	0.031	0.022	0.053
	12.35 p.m.—2.40 p.m..	0.058	0.038	0.094	40. 4	0.032	0.022	0.054
	2.40 p.m.—4. 6 p.m..	0.047	0.035	0.082	42. 7	0.033	0.024	0.057
	4. 6 p.m.—8.55 a.m..	0.480	0.383	0.863	44. 4	0.028	0.023	0.051
	TOTAL.....	0.686	0.522	1.208	43. 2	0.030	0.022	0.052
3rd day..	8.55 a.m.—11. 0 a.m..	0.074	0.058	0.132	44. 0	0.035	0.028	0.063
	11. 0 a.m.—2.15 p.m..	0.093	0.082	0.175	47. 0	0.029	0.025	0.054
	2.15 p.m.—3.40 p.m..	0.045	0.042	0.087	48. 3	0.032	0.030	0.062
	3.40 p.m.—9.50 a.m..	0.508	0.364	0.872	41. 7	0.028	0.020	0.048
	TOTAL.....	0.720	0.546	1.266	43. 1	0.032	0.025	0.057
4th day..	9.50 a.m.—11.10 a.m..	0.063	0.052	0.115	45. 2	0.042	0.035	0.077

It is obvious that the normal variation in the daily and hourly output of creatinine and creatine is very considerable. The hourly rate of $k + k'$ varies from .030 gram. to .077 gram., i.e. $.054 \pm .023$ gram.; a variation of 43 per cent. either way. The average hourly output on a daily basis is much closer, and runs .040 gram., .052 gram., .048 gram., corresponding to the total twenty-four hours' output (interpolated) of .96 gram., 1.25 gram., and 1.05 gram. This daily elimination happens to be fairly regular, only varying ± 13 per cent. or 26 per cent. between extremes, but since the period was so short we cannot assume that this uniformity is general. Indeed, other cases in which twenty-four hours' normal samples were collected at different times from the same sheep on the same diet have shown that daily elimination may be erratic. It may run regular for a few days, shoot up by about one-third, run regular again, drop to two-thirds, rise and run regular again. A direct comparison of Tables XV and IX is of some interest. In the latter, where the sheep was under the same conditions except for the injection of arsenite, we note that on the second, third, and fourth days after injection the daily rate of $k + k'$ elimination averages .043 gram., .052 gram., and .045 gram. per hour, or 1.08 gram., 1.25 gram., and 1.08 gram. per day (interpolated twenty-four hour day). This is actually more regular than in Table XV, so that we cannot say that the arsenic injection has in any way influenced the $k + k'$ elimination. The first day's output of .89 gram. (.037 per hour) in Table IX, the rise to 1.4 gram. on the fifth day, and the fall to .94 gram. (.039 per hour) on the sixth day, do not necessarily mean anything, especially in view of the fact

that the rate of arsenic elimination was very high on the first two days and very low on the sixth.

The ratio of creatine to creatinine, or the percentage of creatine in the total "creatinine plus creatine," runs in much the same fashion in both tables, so that again we cannot trace any influence of arsenic on the indices of endogenous metabolism. The curious fact that in both cases the urine contained little or no creatine during the first few hours of the first day, but that in the course of twenty-four hours it rose to 25-30 per cent. of the total $k + k'$, and in the course of forty-eight hours to over 40 per cent., is not readily explained. It may be chance, or it may conceivably have something to do with the change from loose-box to metabolism crate. The latter is not very probable, but in any case the point has not yet been further investigated. It happens that in both cases the experiment was begun a few hours after transfer to the crate instead of after the usual "pre-day" confinement. If these sheep had been transferred to the crate twenty-four hours before the collection of urine was commenced the anomalous initial absence of creatine would never have been noticed.

APPENDIX A.

Veterinary Research Laboratories,
P.O. Box 593, Pretoria.

DIRECTIONS FOR USE OF THE REMEDY FOR THE TREATMENT OF WIRE-WORMS (AND TAPE-WORMS) IN SHEEP.

POISON.—READ THE DIRECTIONS CAREFULLY.

Number of doses in each tin.—The remedy is supplied in standard quantities each sufficient for 100 single doses, or 50 double doses, for adult sheep. The size of the dose varies with the age of the sheep, so that the standard quantity of 100 adult doses provides 125 doses for two-tooth sheep, 166 doses for lambs between 6 and 10 months old, 250 doses for lambs between 4 and 6 months, 312 doses for lambs from 2 to 4 months old. In ordering the quantities required for a flock it is to be remembered that each animal has to be dosed twice. Stock remedy should be stored in a dry place, and used as soon as convenient after purchase.

TREATMENT OF THE SHEEP BEFORE AND AFTER DOSING.

The period of treatment lasts two and a half days, during which time *no water is allowed*. The sheep are kept without food and water for 12 to 17 hours before dosing and 5 to 9 hours after it. Feeding or grazing is then allowed for two or three hours, but no water. For the following 12 to 17 hours both food and water are again withheld and the second dose is then administered. The procedure on this day is the same as that following the first dose, i.e. nothing is given for 5 to 9 hours, then food without water for two or three hours and again nothing till next morning. The treatment is then complete and the animals are fed and watered as usual. The starvation intervals of 12 to 17 hours before dosing and 5 to 9 hours after it allow of treatment of sheep in batches of such size as can be conveniently dosed in a period of four hours. The following time-table illustrates the treatment recommended:—

1st Day.—*Afternoon*, 1-3 p.m. Remove the sheep from food and water.

2nd Day.—*Next morning*, 6-10 a.m. Administer the first dose.

Afternoon, 3 p.m.-5 p.m. Allow food but no water.

5 p.m. to next morning. Neither food nor water.

3rd Day.—*Morning*, 6 a.m.-10 a.m. Administer the second dose.

Afternoon, 3 p.m.-5 p.m. Food but no water.

5 p.m. to next morning. Neither food nor water.

4th Day.—*Morning*, 6 a.m. Treatment ended. Turn out to food and water.

Treatment of lambs.—For suckling lambs the starvation intervals given above are too long to be tolerated with safety. The time-table for suckling lambs should then run:—

1st Morning.—6 a.m. Separate from the mothers.

10 a.m. Administer the first dose and then starve for 4 hours.

2 p.m. Return to the mothers till evening. Take lambs away from mothers overnight.

2nd Morning.—6 a.m. to 10 a.m. Administer the second dose, starve for 4 hours, and then return to mothers.

It is not advisable to dose lambs and mothers at the same time. All the mothers should be treated first and their lambs then treated about a week later, or vice versa.

After a lamb has been weaned it may be treated as an adult, except that the size of the dose must be regulated according to age.

METHOD OF DOSING.

A dosing bowl, together with standard spoons in sets of five, may be obtained from this Laboratory. The spoons are notched on the handle to indicate the size, the smallest size being marked with a single notch and the largest size being marked with five notches. Markings correspond to age as follows:—

For lambs 2 to 4 months old, the correct spoon has one notch.

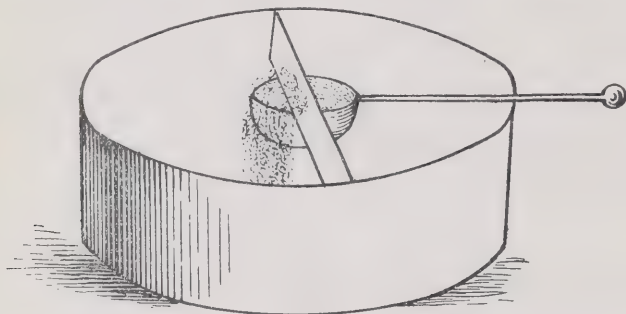
For lambs 4 to 6 months old, the correct spoon has two notches.

For lambs 6 to 10 months old, the correct spoon has three notches.

For 2-tooth sheep the correct spoon has four notches.

For 4-tooth and older sheep the correct spoon has five notches.

The spoons are so constructed that, when filled exactly level, they hold the correct dose corresponding to the age of the sheep. The dosing bowl consists of a small tin with a levelling blade soldered across the top. Sufficient powder, estimated roughly according to the number and age of the batch of animals to be dosed, is emptied into the tin. The correct spoon is selected, dipped into the powder, withdrawn heaped up, and then levelled by drawing across the blade of the bowl, as indicated in the accompanying sketch.



The dose may then be put direct into the mouth of the sheep as far back on the tongue as can be conveniently reached, but it will generally be found more convenient to transfer the powder from the measuring spoon to an ordinary teaspoon for actual dosing. The standard spoons are mainly intended for measuring purposes, whereas the size and shape of an ordinary teaspoon make it more convenient for getting the powder on to the back of the tongue. If the standard measuring spoon is used to dose with, it is apt to get wet with saliva, and this necessitates wiping dry before measuring off another dose, since the powder sticks to a wet spoon and so prevents accurate measurement. A teaspoon, on the other hand, can be kept dry on its upper surface and since it is not dipped into the powder, a wet under surface does not matter. As many teaspoons can also be used as there are men available to carry out the dosing; one man can measure off powder fast enough for three or four dosing operators.

It is important to get the powder as far back on the tongue as possible. If merely placed on the tip of the tongue, the sheep are apt to spit it out. Care must, of course, be taken that the animals do not get any of the powder down the windpipe, and especial care must be taken to prevent accidental administration of two doses to one sheep on the same day. Two doses in one morning may poison the animal. For this reason also it is important to avoid mistakes in selecting the spoons. If a lamb accidentally receives the dose of an adult sheep it is likely to die.

Any unused powder left in the dosing bowl may be put back into the tin and used later, provided it has not become damp. If it has become damp, it will get lumpy and measurement with the spoons will no longer be accurate. Sometimes even the powder in unopened tins becomes slightly lumpy, but such lumps are loose and easily broken up with the spoon.

NOTE.—At a later date an alternative more accurate method of dosing, in liquid form, will be introduced. At the present time, however, suitable implements are not obtainable in sufficient quantity, and only the spoon method is therefore detailed.

REINFECTION OF SHEEP.

Although the treatment cures the sheep of wire-worms for the time being, reinfection takes place when the sheep return to graze over an infected veld. The young wire-worms are found in the grass during the rainy seasons, and during this time a repetition of the treatment once every month should be carried out. This can be done with safety, and in thus repeating the treatment it is possible to gradually clear the farm of wire-worms on a principle similar to that by which periodical dipping frees a farm of ticks.

CAUTION

Since the powder is highly *poisonous*, great care must be exercised in its use and storage.

In dosing for the first time it is advisable to try the effect on ten animals first of all. If this trial is satisfactory, thirty more should be done. If the second trial is satisfactory, the remainder of the flock may be dosed. In this way the sheep-owner can satisfy himself as to the efficacy and safety of the treatment under the conditions prevailing on his own farm.

NOTE.—All farmers are earnestly requested to return all empty tins, boxes, etc., as the utmost difficulty is being experienced in obtaining these articles. Empty returns should be consigned as such to the Director of Veterinary Research, Pretoria North Station, carriage forward.

Price-list.—Dosing bowls, 1s. 6d. each; spoons, 1s. 6d. each, or 7s. 6d. per set of five. With moderate care these last indefinitely. Powder, 1s. per 100 adult doses, sufficient for complete treatment (double dosing) of 50 adult sheep, 83 ten-month old lambs, or 156 young lambs. Post or rail free to any part of the Union.

All laboratory preparations are carefully tested before being sent out, but they are issued solely at the purchaser's risk. The Government cannot accept any responsibility for any losses or accidents which may occur from their use.

APPENDIX B.

UNION OF SOUTH AFRICA.

DEPARTMENT OF AGRICULTURE.

WIRE-WORM REMEDY.

The following memorandum giving certain additional recommendations concerning the Wire-worm Remedy supplied by this Division is issued as a preliminary announcement, and will be supplemented at a later date by more detailed particulars.

Since April, 1917, a considerable amount of additional information has been obtained, and it is now possible to recommend slight alterations in the original instructions, which will have the effect of simplifying the treatment somewhat.

and yet at the same time achieving the desired object. The idea in originally recommending the double dosing during the forty-eight hours of treatment was based on the observation that the double dose kills all wire-worms, but it has now been ascertained that, if a double dose be given twice a year, the dosing to be given during each of the ten remaining months can just as well consist of one dose instead of two. The single dose, when repeated monthly, will kill the young worms, and if double dosings be given at the beginning and end of summer, they will kill any of the old worms that may have lasted over in the sheep during the previous months. Even when sheep are dosed for the first time it is not necessary to give them a double dose, provided the double dose is given at a later date. This point should be particularly taken into consideration when sheep are not in good condition, or when prolonged starvation might do them some harm, or when climatic conditions are unfavourable.

Ewes in lamb.—For practical purposes it is not recommended that ewes heavy in lamb should be dosed with the double dose, although experiments made at the Laboratory on such animals did not prove to be dangerous. However, for the purpose of the practice it would be advisable to arrange matters so that the last dosing takes place about one month before lambing.

Licks.—It has been found that sheep which have regular access to licks are liable to suffer if they are dosed at the same time, and accordingly it should be arranged that during the period of dosing with the wire-worm remedy, they should not be allowed access to such licks.

GOATS.—Goats can also be given the wire-worm remedy, but they should only be given a single dose monthly.

Geilziekte in sheep.—As Geilziekte is probably a sequel of acute worm infection it is also expected that the wire-worm remedy, when given as a single dose monthly, will have a preventive effect.

Ophthalmia in cattle.—When one tin of powder is dissolved in 1 litre of water to which $\frac{1}{2}$ oz. of strong hydrochloric acid has previously been added, it will be found useful for treating cases of ophthalmia (blindness—sore eyes) in cattle. This disease is due to the presence of nematodes, and the solution recommended will destroy the worm. It will, however, not be effective when blindness is too far advanced.

Phials of hydrochloric acid, containing the amount required for 1 litre of water, and a measuring tin of 1 litre capacity can be obtained at a price of 6d. and 1s. 6d., respectively.

Veterinary Research Laboratory,

P.O. Box 593, Pretoria, 19th September, 1917.

APPENDIX C.

THE LIFE-HISTORY OF THE SHEEP WIRE-WORM (*HAEMONCHUS CONTORTUS*).

Abstracted from the Third and Fourth Reports of the Director of Veterinary Research, Department of Agriculture, Union of South Africa.

In the adult stage of wire-worm male and female sexes are recognizable. They can be distinguished by their respective sizes, the female being longer than the male and possessing a peculiar protuberance in the hind third of its length.

Adults.—Both males and females live in the stomach of sheep and other ruminants (cattle, goats, antelopes), and in particular in the *abomasum* or fourth stomach. A sheep once infected with wire-worms, and not treated in any way, may remain infected for at least a year and probably longer. This permanency of infection is of fundamental practical significance, since it is the infected sheep which spreads infection from place to place. The females are fertilized in the stomach and egg-laying continues all the year round, being more pronounced in summer than in winter.

Eggs.—The eggs leave the stomach, pass through the intestines, and are deposited in the faeces. The number of eggs that can be passed by a sheep of outwardly healthy appearance has been calculated to amount to three millions per day, but in badly infected sheep the number is probably much greater.

First larval stage.—Eggs only hatch outside the body. The embryo develops within nineteen hours under optimum conditions of temperature and moisture. In the field the faecal pellets of the sheep provide a suitable medium for hatching out and for subsequent larval growth. The worm leaves the egg-shell and enters the first stage of its larval development, which under optimum conditions may be completed in twenty-four hours. During this time the larva remains within the medium in which it has been growing, develops a new skin, and casts the old one by emerging from its anterior end.

Second larval stage.—Having wriggled out of the old skin the worm enters the second stage of its larval life. It is now very active, feeds freely on the medium, grows rapidly, and undergoes a further change with formation of another new skin. The old skin becomes detached and the worm enters into the third larval stage, called the *mature larva*. In this form it is endowed with longevity and is infective. The eggs and the first two larval stages are non-parasitic and do not infect the sheep.

Third larval stage.—The mature larva now migrates from the medium in which it has grown, wandering upwards and crawling on to the grass in order that it may be taken in by the host and so reach its destination in the stomach. This migration is subject to a number of influences, such as moisture and light. It only takes place when the surroundings are damp, since the worm can not crawl over a dry surface. The most favourable conditions are therefore provided in the rainy seasons, or after heavy fall of dew, or by grass in a vlei. Direct sunshine is not tolerated by the young worms, and if exposed to too strong a light they may be killed. They therefore migrate at night or on rainy or cloudy days. If the sunlight becomes too strong, or the surroundings too dry, they return to the ground and hide in the moist dark soil where they can remain alive for a very long time. Their longevity under suitable conditions may extend up to a year, and this constitutes their most serious menace from the point of view of infectivity. As soon as circumstances are favourable they appear on the grass, are carried to the stomach of the sheep when the grass is eaten, and there continue the cycle of their existence.

Fourth larval stage.—Having reached the stomach of the host the mature larva casts its second skin and begins the first part of its parasitic life. It develops, forms a new skin, casts it, and emerges into the fourth larval stage. It bores into the surface of the mucous membrane of the stomach (*abomasum*), and in doing so causes a slight effusion of blood. This blood coagulates and within the coagulum the larval worm is found. Here it forms its fourth skin. The period taken for the completion of the third and fourth stages is about a fortnight. The differentiation of the sexes occurs in the fourth stage.

Adults.—This fifth and last stage is entered into after the breaking of the fourth skin. Males and females emerge, continue their development, and reach maturity. Fertilization of the female then begins and the process of egg-laying is recommenced, thus completing the life-cycle of the worm. This last stage also occupies about a fortnight, so that, on the average, a month elapses between the entrance of the mature larvae by the mouth and the voiding of new eggs in the faeces. This is of fundamental importance since it determines the period within which repetition of dosing should be made. Under certain circumstances the period between infection and egg-laying may be considerably less than one month, but as a working basis of treatment the month interval has been provisionally adopted.

Summarizing the six stages in the life-history of wire-worm, there must be distinguished three non-parasitic stages and three parasitic stages. Non-parasitic are the eggs, first and second larval stages; parasitic are the third and fourth larval stages, and adult male and female. Transition from non-parasitic to parasitic is effected by the third larval stage or mature larva.

Diagnosis by means of culture.—A practical demonstration of the development of larvae and of their migration can be readily carried out by every farmer by simply placing moist faecal pellets from an infected sheep into a glass jar, screwing down the lid, placing in a cupboard, and watching events from day to day. From the fourth day, providing sufficient moisture is present to deposit a fine dew on the inside of the glass, the larvae begin to migrate upwards and form white slimy tracts ramifying over the surface. If the glass is now

exposed to light it can be observed that the worms return to the faeces and enter them again provided they are still wet. This method may be used by the farmer as a means of determining the extent of infection in his sheep, even before they show the ill-effects of wire-worm infestation.

Practical conclusions.—A living sheep maintains the infection and spreads it so long as adult wire-worms are present in its stomach. An infected pasture remains infected for at least a year, and all sheep grazing over it during this time may become re-infected. Mature larvae are only able to infect sheep under moist conditions of environment, and infection is therefore heaviest during the rainy seasons. The larvae are practically absent from the pasture grass during the dry South African winter months, although they may be present on damp vlei grass from which infection may be picked up all the year round.

Eradication of the worms.—In order to suppress the plague it is necessary to kill the wire-worms in the stomach of the sheep and to clear the farm of mature larvae. Any attempt to keep the hosts (sheep, goats, cattle, antelopes) off the pasture long enough (a year) to starve out the larvae would be impracticable, and it is therefore necessary to adopt some other method. The suggestion therefore is to allow the mature larvae to be picked up by the sheep, but to kill them off in the stomach before they have time to complete their life-cycle and lay new eggs. This can be accomplished by using the wire-worm remedy supplied by the Division of Veterinary Research.

Since the parasitic worm requires about a month to reach sexual maturity and again lay eggs, dosing must be repeated at least once a month during the rainy seasons. After the lapse of a year mature larvae which have not reached their host should practically have died out, and since no eggs have been scattered about by the dosed sheep the farm should then be practically clean. It may ultimately be found advisable to decrease the interval between successive dosings, but in the meantime the interval of one month is recommended as a sound compromise between labour and expense of dosing and a too rigorous interpretation of the minimum period in which the parasitic section of the life-cycle of the worm may be completed. No method can be devised which will keep wire-worms away from the stomach.

Analogy with tick eradication.—It may be explained that the principle underlying these recommendations is the same as that applied to the eradication of ticks. Ticks can not be prevented from attaching themselves to stock, but they can be killed once they have become attached. The stock collect the ticks and the dipping bath kills them before they have had time to complete their development and lay new eggs. Those which are not collected gradually die out, and so the infestation disappears. In the same way the sheep collect the wire-worms and the dosing with wire-worm remedy destroys them. In the case of ticks, destruction at shorter intervals, from three days to fourteen days, according to circumstances, is necessary. In the case of wire-worm it is considered that an interval of a month between successive operations will be sufficient for practical purposes. Just as by means of continued dipping the tick pest is reduced below the danger limit, so by systematic dosing should the wire-worm plague be finally removed.

The Micro-Titration of Arsenic.

BY

HENRY H. GREEN, D.Sc.,

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From the Onderstepoort Laboratories for Veterinary Research.

Pretoria, October, 1917.

The Micro-Titration of Arsenic.*

By HENRY H. GREEN, D.Sc., Biochemist, Division of Veterinary Research, Onderstepoort.

IN the course of some experiments upon the fate of arsenic in the animal organism, it was found necessary to estimate as accurately and speedily as possible quantities varying from one or two milligrams down to about one-fiftieth of a milligram of arsenious oxide. This is a range which is frequently met with in physiological and toxicological work, and yet it appears to be just that range most troublesome to deal with by the methods in general use. For quantities of five to ten milligrams and upwards in a workable bulk of sample there are good gravimetric and volumetric methods. In material containing much organic matter, such as organic arsenic derivatives or organic tissues, quantities of such dimensions may be readily determined by simple Kjeldahl combustion, partial neutralization of the free sulphuric acid by soda lye, final alkalization by sodium bicarbonate, and direct titration by iodine. The combustion of the organic matter is associated with complete reduction of arsenic. A useful note on this method has been recently given by Ewins,[†] in which it is shown that for organic compounds of arsenic the process is extraordinarily simple and accurate.

We had ourselves made frequent use of such a method in cases where the amount of arsenic was known to be high, but had regarded it as unsuitable in cases where the arsenic fell to the region of a milligram. In checking the accuracy of the direct iodometric determination of arsenite, and titration of arsenate after reduction with thio-sulphate by Chapin's method, in foul cattle dips containing much excreta, the direct titration of total arsenic after simple Kjeldahl combustion had been found very convenient. But for the determination of arsenic in stomach contents or urines it had not been found satisfactory, owing to the fact that a blank determination on arsenic-free material frequently gave an appreciable iodine reading. When Ewins' paper appeared we repeated this observation and found, for instance, that 50 c.c. of horse urine gave a blank equivalent to 0.4 mg. As_2O_3 . Direct methods therefore, which are accurate when the amount of arsenic to be determined is considerable, may break down when the quantity falls below a few milligrams. Below this it appears to be an

* Read before the South African Association for the Advancement of Science. July, 1917.

† A. J. Ewins: "Estimation of Arsenic in Organic Compounds." *J.C.S.*, Dec., 1916.

almost universal practice to bring over the arsenic in the form of arsenuretted hydrogen and to apply either the Marsh test or one or other modification of the Gutzeit colorimetric determination. To obtain accurate results by the Marsh test, to discriminate, for example, between 0.6 and 0.8 mg. or 0.06 and 0.08 mg., requires very careful manipulation. Mirrors may be very deceptive unless procured under absolutely identical conditions, and the trouble associated with the heating of the Marsh tube and rigorous regulation of other conditions is unwelcome. It is probably for such reasons that the Gutzeit colour test has largely replaced the Marsh.

For exceedingly small quantities of arsenic both Marsh and Gutzeit tests are of course beyond cavil, for the simple reason that they are the only methods available, and that as the absolute amount of arsenic diminishes relative accuracy of determination becomes less important. The analyst, for example, is rarely expected to do more than distinguish between .01 and .02 milligrams or .001 and .002 milligrams in a workable quantity of material. These limits represent for most practical purposes a "trace," and a relative error of 100 per cent. is of no consequence. An error of 20 per cent. may be significant, however, if the quantity is in the region of one milligram, and it is just here that the Gutzeit or Marsh is most open to criticism. The disadvantage of all such colorimetric methods is that, no matter how much arsenic is actually present, the final solution upon which the determination is carried out must be diluted down to render it comparable with the standards. Working with mercuric halide papers most analysts aim at solutions such that not more than .05 mg. is actually taken for stain production, and in general prefer to work with stains corresponding to about .02 mg. Above this the colours are too dark to match with accuracy.

The result is that, whatever be the absolute amount of arsenic, the relative percentage error remains high. The accuracy turns wholly upon a rigorous observance of identical conditions in analysis and in control, and the process requires considerable care and judgment. The mode of distribution of the colour stain over the mercuric chloride paper varies with the rate of evolution of hydrogen, and it is very difficult to compare stains derived from identical quantities of arsenic unless the rate is carefully controlled. In an electrolytic generator this may be easy of accomplishment, but wherever zinc and acid are used (the more general practice) the apparatus requires careful attention. The personal equation in reading stains is considerable, and working with material of known arsenical content we have experienced at one date a tendency to get consistently high results, and at another date consistently low results. Where the number of determinations carried out at one time is great this factor is less important, colour judgment and rigorous observance of conditions becoming almost instinctive, but for occasional analyses the personal factor is considerable.

A further serious disadvantage of colorimetric methods is that in working with a material whose arsenic content is not even approximately known a preliminary determination has to be carried out merely for the sake of fixing the appropriate dilution for the final estimation. One may be quite unaware whether the arsenic in a given combustion residue amounts to 2 mg., 0.2 mg., or .02 mg. In the last case an appropriate dilution is obtained by taking the whole

residue. In the other two, dilutions of 1:10 and 1:100 respectively are suitable. Furthermore, although permanent preserved standards are very useful for arriving at approximate results, most analysts will feel safer with at least one fresh control stain. Dealing with an unknown material three determinations may then be required to yield an accurate result—one to fix dilution, one to obtain a suitable stain, and one fresh control stain derived from a quantity of arsenic similar to the unknown. This is admittedly troublesome.

That we are not alone in our experience of the irregularity of results obtainable by the colorimetric methods is evidenced by reference to the general literature, and in particular to recent official trials of the method in America. In the recent report of Loomis^{*} on heavy metals in foods, the results of co-operative work amongst the American agricultural chemists is given. In a trial in which eight analysts reported upon the same two samples, a sweetened gelatine solution containing 7 mg. As_2O_3 per kilo, and a fruit syrup containing 4 mg. per kilo, we have the following results:—

Method: Smith modification of the Sanger-Black colorimetric process, using mercuric halide paper.

Analyst.	Sweet Jelly containing 7 mg. As_2O_3 per kilo.				Fruit Syrup containing 4 mg. As_2O_3 per kilo.			
				mg.				mg.
C. C.	6.0	1.0
W. S. A.	7.0	4.0
E. H. B.	5.0	4.0
W. W. K.	7.6	3.2
E. O. A.	5.0	6.0
				6.0				6.0
				6.0				8.0
T. F. P.	6.6	4.0
C. R. S.	6.0	4.5
				6.5				—
				6.5				—
H. E. W.	6.0	5.0
Maximum	7.6	8.0
Minimum	5.0	3.2
Variation	2.6	4.8
Average	6.2	4.9

It will be seen that in the returns of eight analysts the variation is 2.6 in 7, or 37 per cent. in one case, and 4.8 in 4, or 120 per cent. in the other. Also, that in the triplicate returns of one of the co-operators the average figure for the jelly is 5.7, or 19 per cent. too low, and for syrup 6.7, or 68 per cent. too high; i.e. although the jelly actually contains 75 per cent. more arsenic than the fruit syrup, it is returned as containing 15 per cent. less. The results C.R.S. are those of the originator of the modification of the process, and may therefore be taken as the most experienced: they show a range of error of 20 per cent.; 8 per cent. too low for the jelly and 12 per cent. too high for the syrup. Even when the average results of eight independent

* *Journ. Assoc. Offic. Agric. Chemists*, Vol. I, No. 2, Part II, p. 245. August, 1915.

analysts, using exactly the same method, are considered, the jelly is returned as 11 per cent. too low and the syrup as 22 per cent. too high—an error range of 33 per cent. It may be further mentioned that in the returns of the same analysts using "other laboratory methods" the results are just as divergent—W.S.A. reporting 10 mg. for the jelly and E.H.B. reporting 4.6 mg. as against the theoretical 7 mg.

Nevertheless, the results, taken altogether, are regarded as "very good" by the referee, and the data may therefore be taken as showing that the accurate determination of small amounts of arsenic is by no means simple.*

* In the interval between the date of writing this note and the date at which it reached the printer the results of further co-operative work in America have reached us—'Trenhard': "Report on Heavy Metals in Foods." *Journ. Assoc. Offic. Agric. Chemists*, May, 1917, Vol. III, No. 1, p. 45.

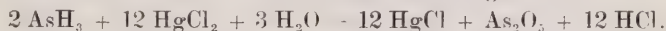
Co-operative Results on Arsenic.

ANALYST.	SAMPLE 1.	SAMPLE 2.
	Sugar Solution containing 1.4 mg. As_2O_3 per kilo.	Gelatin Solution containing 12.0 mg. As_2O_3 per kilo.
	Mg. As_2O_3 found.	Mg. As_2O_3 found.
C. L. B.....	1.9 1.7	14.3 13.3
V. B. B.....	1.3	9.0 9.8
L. D. E.....	1.8 2.0 1.6	10.0 12.0 16.0 15.0 *20.0
L. F.....	0.8 0.8	10.2 10.1
S. G.....	*4.0	*20.0
L. E. H.....	*2.2	12.8
L. A. S.....	*0.6	*5.8
C. C. S.....	1.0	*5.0
G. W. T.....	*0.4 *0.2	*6.0 *5.0
E. L. P. T.....	1.8 1.8 1.4 1.4	10.0 12.0 12.8 13.2
H. A. W.....	*0.3	*2.5
Average.....	1.4	11.1
Variation.....	0.2-2.2	2.5-20.0
Average, excluding results marked *....	1.5	12.0
Range.....	0.8-1.8	9.0-16.0

These results illustrate very forcibly the difficulties encountered in the colorimetric estimation of arsenic in foods. Excluding the worst results marked with an asterisk by the referee, the returns vary from 0.8-1.8 mg. or 57-130 per cent. of the theoretical in one case, and 9.0-16.0 mg. or 75-133 per cent. in the other case. If we include the asterisked data, the highest results are *ten times* as high as the lowest. The comment of the referee is: "In view of the small amount of arsenic present, many of the results are fairly satisfactory, although there is great discrepancy among some. There still appears necessity for more careful regulation of the conditions of operation."

In general practice the error range indicated may not be of great practical consequence when the amount of arsenic is so low as 7 mg. per kilo. But in physiological work where, for instance, an attempt is made at following the rate of elimination of known quantities of absorbed arsenic, a percentage error of the magnitude in some of the results quoted above would be highly undesirable—might even invalidate the whole work.

We hope to show that by the micro-titration method outlined in this note it is possible to attain a much closer degree of accuracy than is generally attained by colorimetric methods, whenever the quantity of arsenic available for determination is of such size as necessitates dilution for colorimetric comparison; in other words, whenever a quantity of raw material containing one-twentieth of a milligram and upwards of arsenious oxide can be comfortably managed in the preliminary process of wet or dry combustion. The method itself is not novel in principle, being simply a modification of the Mai-Hurt-Gutzeit process in which the arsenic is brought over as arsenuretted hydrogen, absorbed in silver nitrate solution, and then titrated. At the time the method was being tested we were unaware that a micro-titration process had already been worked out by Smith,* but we have since procured a copy of the paper concerned and find that Smith has utilized the reaction between arsine and mercuric chloride as the basis of a titrimetric method. The reaction itself is given as:—



The arsine is absorbed in dilute mercuric chloride, and (a) the conglomerate treated with excess of potassium iodide, excess of N/10 or N/50 iodine to dissolve the precipitate, and then titrated back with standard thiosulphate; or (b) preferably converted into pure calomel by boiling, and weighed as such.

The results reported are good, but for some reason or other were not made the basis of co-operative trial in the report of Loomis, already mentioned—though it appears to us that the quantities of arsenic in the jelly and syrup were susceptible of determination by the method.

The process we ourselves have now adopted, with satisfactory results in an extensive series of experiments, is, we think, even simpler, and therefore worth recording.

In the modified Gutzeit of Mai and Hurt the reaction involved is represented by the familiar equation of the textbooks:—



A known quantity of silver nitrate is taken, the precipitated silver filtered off and residual silver nitrate titrated with thiocyanate. In the modification proposed here it is the arsenious oxide itself which is titrated and not the silver. The advantage of this is that estimation is direct, that it allows of a more delicate end-point (starch), and that no accurately measured amount of silver nitrate, varying with the amount of arsenic expected, need be taken. The sensitiveness aimed at is greater than the original process of Mai and Hurt, and the manipulation is easier. The AsH_3 from the generator is simply fixed in an adequate amount of dilute silver nitrate contained in a test-tube.

* C. L. Smith: U.S.A. Dept. of Agric. Bureau of Chemistry Circular No. 102.

or series of test-tubes, no elaborate absorption bulbs being necessary. The whole contents are then rinsed into a small flask with the minimum of water (a few c.c.), a little sodium bicarbonate added, and then sufficient excess of solid potassium iodide to dissolve up the precipitated carbonate, arsenite, and iodide, of silver.

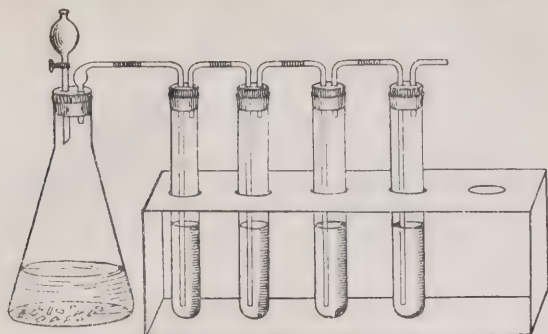
A little clear starch solution (filtered) is then added and N/495 iodine run in to end-point. 1 c.c. = 0.1 mg. As_2O_3 . The end-point is brownish-violet instead of the usual starch-iodide blue, and is sensitive to two drops of iodine, equivalent to one-hundredth of a milligram of arsenious oxide. If the amount of arsenic is very small the titration may be conducted in the absorption test-tube itself, with even more dilute iodine, and the end-point determined by looking down the tube against a white background. Except where the amount of arsenic is considerable it is unnecessary to filter off the precipitated silver since the latter is flocculent and does not seriously obscure the end-point.

Before testing the accuracy of the absorption of small amounts of arsine by silver nitrate, the accuracy of the titration itself was tested. Using thousandth normal iodine with thousandth normal sodium arsenite in presence of excess of silver nitrate, small adequate amounts of bicarbonate, large excess of potassium iodide, and a few drops of clear starch solution, it was found as easy to titrate hundredths of milligrams as it is to titrate milligrams with deci-normal reagent.

In first testing the absorption of arsine by dilute silver nitrate the usual precautions concerning rate of passage of gas from generator were observed, but it was soon found that the absorption was so rapid and complete that only the most crude precautions were necessary.

A series of hard glass test-tubes (about 6 in. by $\frac{3}{4}$ in.) were fitted with rubber corks and ordinary glass connection tubing. The first of these was filled to a depth of about 5 cm. with 5 per cent. lead acetate solution, while the remainder were filled to the same depth with dilute silver nitrate—a few c.c. N/10 diluted with water. On attaching to the Marsh generator it was found that however fast the gas was passed, short of blowing the contents of one tube into the next, absorption was practically complete in the first two or three tubes. Only when the amount of arsine was high and the gas current very rapid, could any appreciable amount be swept into the fourth tube. With amounts of arsenic of 1 mg. and less, and a rate of gas passage of about 200 c.c. per minute, the third tube remained practically clear. Using a generator flask of 150 c.c. capacity with 1 mg. As_2O_3 , it was found easily possible to bring over and quantitatively absorb 70 per cent in two minutes and 95 per cent. in ten minutes—80 per cent. being retained in the first tube, 15 per cent. in the second, and only a trace in the third. Using a larger generator the time required is naturally a little longer. This great rapidity of absorption is very useful in enabling the analyst to cut down the working time of a determination and avoid the necessity for any very careful control of the rate of evolution of hydrogen. When pure sodium arsenite is introduced into the generator, and no foreign salts are present to retard liberation of arsine, an estimation can be run through in twenty minutes. Any arsenic not brought over in this time only comes over very slowly thereafter—may indeed never be completely recovered, and it is best allowed for by a small empirical correction.

The apparatus, as indicated by the diagram, is very simple:—



A small wooden rack serves to hold the hard glass test-tubes. The first tube containing 5 per cent. lead acetate solution serves to retain any sulphuretted hydrogen passing over with the gas, but unless the acid in the generator is unnecessarily strong, and the contents become unduly heated, the amount of this impurity is not large. Undue heating can easily be avoided by slipping a vessel of cold water under the generator. The first silver nitrate tube contains solution in concentration of anything between $N/20$ and $N/100$, according to the amount of arsenic expected. The silver nitrate in the second tube should be still more dilute (say 1 c.c. $N/10$ diluted with water in the tube to a depth of about 5 cm.), since the less total silver salt used the smaller is the amount of potassium iodide required in the subsequent titration. The third tube is rarely needed, but is put on as a check on possible loss of arsine by too rapid evolution of gas from the generator. The concentration of silver nitrate may be again made high ($N/20$ to $N/50$), so that it remains as a guard in case the amount of arsenic present is very much above expectation. A fourth tube may be held in readiness to be attached if required. The delivery tube of the generator is either made of wide glass constricted at the end to take the rubber connection tubing, or is widened into a bulb at the bend so as to prevent the spray from the generator from lodging in the form of drops and being carried over into the lead acetate tube. The zinc, in granulated form for preference, is placed in the flask and the apparatus connected up. The solution in which the arsenic is to be determined is then washed in through the tap funnel, and further acid, usually 1:3 sulphuric, is added in amount sufficient to induce a rapid but not excessive evolution of hydrogen. The rate is easily controlled by the addition of more acid or more water, and by cooling the generator or allowing it to warm up, but, as already mentioned, it will be found that no particular trouble need be taken. Half a dozen or more generators may be attended to at once if the number of determinations to be executed is great. After twenty to forty minutes' vigorous passage of gas the silver nitrate tubes are detached and a fresh tube put on if it is desired to control further evolution of residual traces of arsenic. The titration is carried out as indicated above—usually by washing the first two tubes into a small flask with a few c.c. of water, and ignoring the third. The less wash-

ing water used the better, since increase in the volume of liquid involves increase in the amount of potassium iodide required to keep the silver salts in solution. If during titration the dilution rises to the point of reprecipitation of silver iodide, a few more crystals of potassium iodide may be added to clear up the solution. A blank determination should, of course, be run to determine the purity of the zinc and to allow for the drop or two of iodine solution required to produce a distinct starch end-point. It is hardly necessary to emphasize the importance of a suitable grade of zinc, since this point is fully discussed in the textbooks and the recent literature, but it may be recalled that it is not sufficient that the zinc be arsenic-free. It must also be free from metals which retain arsenic and prevent complete liberation of arsine from the generator.

Arsenic-free zinc, which contains traces of cadmium or tin, is superior to chemically pure zinc, since it does not then require to be sensitized for use. Needless to say, an electrolytic generator with cadmium or lead electrodes is to be preferred as avoiding all such difficulties and allowing of any desired gas current. Unfortunately, however, such electrolytic generators were not at our disposal, and prevailing conditions prevented the procuring of them.

In obtaining the following illustrative results, a generator flask of 150 c.c. capacity was used, with a granular zinc which required no catalyst. Ten to twenty grammes of zinc were used, covered with water, known amounts of standard sodium arsenite added, and the rate of evolution of hydrogen controlled by adding 25 per cent. of sulphuric acid through the tap funnel. In all cases the silver nitrate tubes were disconnected after twenty to twenty-five minutes' vigorous bubbling through of gas. Only the first two tubes were united for titration—the third remaining clear throughout. For the last two columns it was only necessary to titrate the first tube (about $N/100 \text{ AgNO}_3$), and titration was carried out in the tube itself.

As_2O_3 taken.	2 mg.	1.5 mg.	1.0 mg.	0.5 mg.	0.3 mg.	0.2 mg.	0.1 mg.	0.05 mg.
C.c. $N/495$ Iodine								
(a)	19.5	14.4	10.0	4.9	2.8	1.9	1.0	0.55
required (b)	19.7	14.4	9.8	4.7	2.9	1.9	0.9	0.50
(c)	19.4	14.2	9.9	4.8	3.0	2.1	1.1	0.45
Average ...	19.5	14.3	9.9	4.8	2.9	1.96	1.0	0.50
Blank ...	0.1	0.1	0.1	0.1	0.1	0.1	0.05	0.05
As_2O_3 recovered, mg.	1.94	1.42	0.98	0.47	0.28	1.86	0.95	0.045
Percentage of theory	97	95	98	94	93	93	95	90

These data suffice to show that in so far as the quantitative absorption and titration of the arsenic is concerned, the use of silver nitrate and iodine leaves little to be desired. A recovery of 98 per cent. with one milligram and 95 per cent. with one-tenth of a milligram is undoubtedly good. The results are uniformly a little low, owing to retention of traces of arsenic in the generator. It is possible that with an electrolytic generator the data would be even more perfect. After disconnecting the silver nitrate tubes traces of arsine could still

be detected by attaching a small cap of mercuric chloride paper to the exit connection, but no better results were obtained by running the determination for an hour instead of twenty-five minutes.

It will be generally admitted that results such as these are not easily obtained by comparison of Marsh mirrors or Gutzeit papers. The contention therefore is that wherever amounts of arsenic above one-twentieth of a milligram have to be dealt with, titration should always be adopted, and the whole object of the present note may be regarded as a plea for this procedure. The difficulties of procuring even stains and the uncertainty of matching them by eye are eliminated, and judgment is replaced by fool-proof burette reading. The advantage of this is that if, in practice, any determinations are obviously at fault, the source of the error is easier to find. Failure to bring over the arsine is readily detected, and the blame attached to the zinc in use, or to the presence of arsenic-retentive salts in the combustion residue, as the case may be. Losses dependent upon the mode of preliminary treatment of material are more readily detectable and numerically expressible, and are not confused with a stain-matching error. If any correction of data has to be made it is done consciously and not unconsciously as is usually the case in colorimetric tests, where "error" is automatically allowed for by embodiment in control stains obtained under strictly parallel conditions.

And some sort of correction is frequently necessary in determining arsenic. In urines, for example, using either the magnesium nitrate dry combustion, or the nitric sulphuric wet combustion, the results are invariably too low. But they are consistently low, and agreement between duplicate determinations is fairly good—to within ± 5 per cent. As a rule the relative shortage from the theoretical increases as the absolute quantity diminishes. On 50 c.c. of sheep urine containing 2 mg. As_2O_3 , added as arsenite, only 1.8 mg. may be recovered after combustion—a shortage of 10 per cent. When the added arsenic is 0.5 mg. only, .03 to .04 mg. may be recovered—an error of over 20 per cent. By carrying out a series of determinations upon arsenic-free material similar in nature to that under routine examination, and plotting added arsenic against arsenic recovered, a correction curve may be constructed by subsequent use of which considerably enhanced accuracy may be obtained. In general analytical practice, however, particularly with food-stuffs or toxicological material, an error of one-tenth to one-fifth is usually of no importance, and correction may be omitted.

The shortage from the theoretical seems to arise through retention of arsenic in the generator, and might be ascribed to the presence of foreign salts, such as these of iron, in the combustion residue. It appears highly probable to us that the use of an electrolytic generator would obviate the necessity for any correction, and we hope to test this point as soon as facilities (at present unavailable) permit. At any rate, we are satisfied that the error arises in failure to bring over the arsine quantitatively and not in the absorption and titration advocated in this note. Such an error would, therefore, be encountered in *any* method dependent upon separation of arsenic as arsine.

When the zinc and acid generator is used (the commoner case) each analyst should prepare a correction curve for himself, adhering throughout to the same method of preliminary combustion of material, in order to satisfy himself as to his own working limits of error.

It is claimed that in this laboratory, working with stomach contents, faeces, urines, tissues, etc., and using a correction curve, a degree of accuracy within ± 5 per cent. of the truth is obtained on all quantities above one-tenth of a milligram. Between one-tenth and one-twentieth of a milligram the error is not much higher, although at the lower limit it is frequently ± 10 per cent. This compares very favourably with the results obtained by any other process, as judged by the limits of error shown in co-operative trials. For quantities below one-twentieth of a milligram, it is still possible to carry out titration, especially if smaller absorption tubes and more dilute iodine be used, but as the quantity of arsenic diminishes the advantage so gained disappears, and it becomes preferable to fall back on colorimetric modifications of the Gutzeit test—the Smith adaptation of the Sanger-Black modification, using mercuric chloride or mercuric bromide test papers, being apparently the simplest.

In regard to the preliminary destruction of organic matter in samples, we regard dry combustion, after addition of or wetting with magnesium nitrate, and wet combustion with nitric and sulphuric acids, as equally satisfactory, but in general find dry combustion more convenient and expeditious wherever it is necessary to work up considerable bulk of material in order to secure a suitable ultimate yield of arsine. We have not experienced the difficulties met with by some workers in effecting reduction of arsenate to arsine by zinc and sulphuric acid, and have not found it necessary to effect preliminary reduction to arsenite. Vigorous evolution of hydrogen (allowing the generator to warm up somewhat) was found to bring off arsine as quantitatively from arsenate as from arsenite. Preliminary reduction of arsenate by stannous chloride and potassium iodide is advocated in the report of Loomis already referred to, but we have not found this necessary when no stress is laid upon maintaining a slow and steady current of hydrogen from the generator, i.e. when the titration method is used.

SUMMARY.

The difficulty of determining small quantities of arsenic in physiological material with any real approach to percentage accuracy is emphasized, and it is pointed out that for quantities ranging from two or three milligrams down to one-twentieth of a milligram in a workable bulk of material, a micro-titration method is more serviceable than the commonly used Marsh mirrors or Gutzeit papers. A method is described in which the arsenic is brought over as arsine, collected in dilute silver nitrate, and directly titrated with $N/495$ iodine (1 c.c. = 0.1 mg. As_2O_3) after addition of bicarbonate and sufficient potassium iodide to keep all excess silver salt in solution. Comparison is made with the reports of referees in the most recent trials of methods favoured by the Association of Official Agricultural Chemists in America, and it is maintained that micro-titration is more reliable and more rapid than colorimetric determination; that it requires less personal attention to detail, and is applicable in a great many cases where most chemists now adopt a modified Gutzeit method.

The Effects of Arsenite of Soda Dipping Fluids on Working Oxen.

BY

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The Effects of Arsenite of Soda Dipping Fluids on Working Oxen.

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THE regular dipping of cattle in solutions containing arsenite of soda has become general in properly conducted farms throughout South Africa and while the effect on young stock and breeding animals has left little to be desired, on oxen to work, the system has, in most cases, been followed by a falling off in the working capacity of the animals. The effects of the dip are produced by absorption through the skin of the arsenical solution which causes considerable interference with the respiratory mechanism, giving rise in severe cases to laboured respirations and profuse sweating when animals are put to strenuous work. The degree of respiratory distress has been noted to depend on several factors:—

- (a) Previous regular dippings while young give rise to condition of tolerance and when these animals are put to work the symptoms produced are not so marked. This tolerance becomes more pronounced as the animals grow older.
- (b) Climatic conditions exert a marked influence. The most marked symptoms being set up on hot days on which there is not much wind.
- (c) The maximum effects are not produced immediately after dipping, but after an interval of a day or two.

In order, therefore, to ascertain with accuracy the conditions under which these effects were produced and to devise, if possible, a system of dipping which, while the lethal effect on the ticks was not interfered with, would cause least distress during work, the following experiments were undertaken at this Laboratory:—

Ten three-year-old oxen were dispatched from Pretoria, having been inoculated against Redwater and Gall-sickness nine days previous to dispatch. These animals had not previously been subjected to regular dippings. Five oxen were taken, Nos. 87, 88, 89, 90, and 96, and dipped at five-day intervals in Laboratory dip, and the remaining five animals, Nos. 91, 92, 93, 94, and 95, received dipping at similar periods in a dipping tank containing water only. These former animals were called the "Dipped lot" and the latter the "Control lot," and will be referred to as such during this report. The Laboratory dip used in the experiments contained a specified percentage of arsenite of soda, plus the usual quantities of paraffin and soft soap recommended for use in the five-day dip, namely 5½ lb. soft soap and 2 gallons of paraffin per 400 gallons. The dip was kept at the required strength by frequent tests for the arsenic content during the dipping periods. The animals on arrival were in rather low condition, and some time was allowed to elapse before commencing to work them, in order to allow the animals to improve in condition, and to recover completely from the effects of the Redwater and Gall-sickness vaccination. Grass at this time—and for some months afterwards—was very poor and so an additional ration was given to the animals.

By an arrangement with Mr. Dales, manager for the Wattle Syndicate, Town Bush Valley, the oxen were worked by his boys in cultivating areas of the wattle plantations adjoining the Laboratory. In this way it was possible during the experiment to keep the animals under constant supervision. Visits were made at short intervals to the area in which the animals were working, and the condition of the animals while in the yoke observed and recorded.

EXPERIMENT A.

Dipping of the animals was commenced on 11th April, 1914, and the animals were put to work on 13th April, 1914. The dip used for the "Dipped lot" contained .123 per cent. arsenic, and dipping was continued in this strength dip every five days until 5th of June, 1914, during which time the animals received eleven dippings. The effect of the dip on ticks in either the "Dipped animals" or "Controls" during this period was not observed.

Notes on the Experiment.—On the fourth day after the first dipping one of the "Dipped lot," ox No. 96, died about noon. The day was very hot, and the animals on being outspanned immediately went to the spruit to drink; this ox died suddenly on leaving the spruit, having—according to the boys who were working the oxen—shown no previous symptoms. A post-mortem was made, but no lesions were found to which death could be attributed. It is, therefore, impossible to say definitely whether the dipping was the actual cause of death, but from information regarding similar cases received from farmers subsequently, I am of the opinion that the exciting cause of death was the effect of the arsenic in conjunction with strenuous work on a very hot day before a tolerance to the drug had been set up.

On 26th April, 1914—the day of the fourth dipping—two of the "Dipped lot" showed marked respiratory distress during work. Lachrymation, and a desire to lie down in the yoke was also observed. No further symptoms, which could be attributed to the effect of the dipping were noted during this period. Two of the "Control" oxen were observed on the 11th May, 1914, to have swelling in the hind limbs, No. 91 in the region of the semi-membranosus muscle, and No. 95 in the region of the gastrocnemius muscle. In each case in the off hind leg. These swellings were hard and painless, and evidently due to injuries received on entering the dipping tank. A similar swelling, but of a painful nature developed in the shoulder-joint region of ox No. 94, and so this animal was taken off work and placed under treatment. All of these animals recovered.

Results.—The results of this experiment showed that a dip containing this strength of arsenic could safely be used in oxen in work after the oxen had become tolerant. As the observations were being made at a time when the climatic conditions were most favourable for working animals, viz. during the colder months; this statement cannot, however, be taken as final. Although no tick counts were carried out during this period, it was considered by observation on the tick infestation of the animals, that dipping in this strength of arsenic is insufficient to effectually destroy ticks. In order to prove if this was the case, the experiment was continued with a similar strength dip for a further period of thirty days—six dippings—and a daily count made of all ticks alive and dead found in the brush and under the tail.

As a result of these observations it was found that in the brush 46.3 per cent., and under the tail 56.3 per cent. of ticks were killed. On reference to Table A (I), it will be seen that the actual number of ticks on which these percentages were made are somewhat small, especially in the case of ticks attaching to the brush, but this is explained by the fact that the experiment was conducted at a time of the year when the ticks were—to a great extent—quiescent. The results of similar observations on the “Control” lot will be found on reference to Table A (II). Here it will be seen that the percentage of the dead, missing, or engorged ticks is very much lower, by 33 per cent. in the brush and 12.2 per cent. under the tail. As in Table A (I), the number of ticks attaching to the brush is so small that it would be impossible to work out a true percentage, and that given does not, in my opinion, represent a true mortality in either case.

Effect on Oxen in Work.—Observations made on the oxen in the yoke from 5th June, 1914, to 30th June, 1914, while being dipped in dip containing .123 per cent. of arsenic, showed that no respiratory distress became evident in excess of the normal. Loss of condition occurred in all the animals, but this was not more marked in the “Dipped lot” than in the “Controls.”

Conclusions.—Dipping of oxen in a dip containing .123 per cent. of arsenic does not cause any distress to the animals when pulling in the yoke after a short period, during which a tolerance is established. This strength of dip, however, has not sufficient lethal action on ticks to recommend it for use in practice.

EXPERIMENT B.

A further test was now begun with a dip containing an increase of .01 per cent. arsenic, viz., .133 per cent. The animals used in the previous experiment were utilized, four being dipped in arsenite solution every five days, and the remaining four dipped in plain water. The experiment was carried out from 5th July, 1914, to 3rd August, 1914, during which time the animals received six dippings.

Lethal Effect on Ticks.—Owing to the climatic conditions during this experiment, ticks were not much more numerous than in the former experiment. The resulting percentages of ticks killed can only be taken as approximately correct. The lethal action was much more strongly marked than in the experiment with .123 per cent. arsenic, and this was not only observed in animals under experiment, but also in other animals being dipped in the tank. On reference to Table B (I), it will be noted that in the “Dipped lot” the destruction of the ticks was as follows:—In brush 37.3 per cent.; under the tail 61.5 per cent. The death-rate of the ticks on the “Control lot” [Table B (II)] for the same period was as follows:—In brush 11.3 per cent.; under tail 9.8 per cent. In the latter animals a number of ticks engorged, and were either collected or dropped off accidentally. These have been included under the heading of “missing and engorged” in the table.

Effect on the Oxen in Work.—The effect of this strength dip (.133 per cent. arsenic) on the oxen in work was very marked. The severity of the symptoms varied with the length of time after dipping and the daily atmospheric temperature. The symptoms shown were as follows:—Head carried low, mouth partly open, tongue in some cases protruding, marked salivation, pulling very badly in the

yoke, respirations very much increased—short and jerky—and very marked distress present. The condition seemed to become more aggravated about five minutes after the animals had stopped pulling, and the symptoms of distress continued for about fifteen minutes. The breathing of the four “Controls” was not much interfered with, and although the pulling accelerated the respiratory movements temporarily, as soon as the animals were allowed to stand the condition rapidly diminished, and it was very easy to say definitely which was “Dipped lot” and which were “Controls” by the walk, the respiratory movements, the carriage of the head, and, later, the general willingness to pull of the latter animals. As a result of many observations during this dipping period, it was ascertained that the effect of the dipping did not reach its maximum until the third day after dipping. Comparatively little respiratory distress was observed in the “Dipped lot” on the day of dipping, or the following day. The temperature had also a very marked influence. On cool days the amount of respiratory distress in the dipped animals was not so marked as in the warmer days.

On one occasion—for a period of three days—during this dipping period, one of the “Controls” was observed to hang back in the yoke, and to show considerable respiratory distress. As the other “Controls” remained normal, and showed no such symptoms on this occasion and as no other symptoms were apparent, it was concluded that the condition in this ox was due to some temporary systematic derangement. A high atmospheric temperature was registered just at this time, and it is possible that this also might have had some influence.

Conclusions.—A solution of sodium arsenite containing .133 per cent. arsenic is of sufficient strength to kill the majority of ticks which attach, and dipping in this, carried out at five-day intervals, prevents infestation of animals by ticks in large numbers. Its effect, however, on oxen in work, is so drastic that it could not be recommended in practice for these animals.

EXPERIMENT C.

Owing to the severity of the reaction in oxen in work with a dip containing .133 per cent. arsenic it was decided to reduce the strength of the solution to .128 per cent. arsenic, and to dip the animals as before at intervals of five days. This experiment was commenced on 4th August, 1914, and continued until 9th September, 1914, the animals during this period receiving seven dippings.

General Observations on the Experiment.—It was noticed soon after the commencement of this experiment, that all the animals were losing condition. This was probably due in part to the onset of warmer weather and in part to harder work, as a heavier plough was being used than had previously been in use. The loss of condition was most marked in the “Dipped lot,” although in the “Control lot” the loss was also considerable. It became necessary on the 24th August, 1914, to discontinue work temporarily in order to allow the animals to graze continually, and so pick up in condition. A week’s rest was given, and on 30th September, 1914, the condition of all the animals had so much improved that work was recommenced.

Effect of .128 per cent. Arsenic on Ticks.—The number of ticks attaching to the animals during this experiment was much in excess

of that during previous observations. On reference to Table C (I), it will be seen that in the "Dipped lot" 25.4 per cent. of ticks which attached to the brush, and 56.2 per cent. of ticks which attached under the tail were killed, while in the "Controls" [Table C (II)], the death-rate was 6.6 per cent. in the brush, and 3.0 per cent. under the tail during the same period. As in the former cases, ticks on these latter animals which engorged and were collected or dropped off accidentally are included under the heading "missing and engorged" in the table.

Effect on the Oxen in Work.—Marked symptoms of distress were observed in three of the "Dipped lot" (Nos. 87, 88, and 89), on the second day after the first dipping. These animals showed salivation, lachrymation, and very much increased respiration. Similar symptoms were observed in all four dipped oxen up to the date on which work was discontinued, i.e. 24th August, 1914. The condition of the animals, and the heavy work in which they were engaged, made it difficult to say how much of this distress was due to the dip. I was of the opinion that the effect of the dip was aggravated by these above-mentioned factors, and for this reason it was decided to give the animals a week's rest. This opinion was confirmed on the animals again commencing work, when it was observed that very little respiratory distress became evident until the close of the experiment.

Conclusions.—On reference to Table C (III), it will be observed that there was a steady increase in the number of ticks present in the "Dipped lot" up to the tenth dipping. The lethal effect on ticks, therefore, of a dip containing .128 per cent. of arsenic cannot be looked upon as sufficient for practical purposes. The observations on the effect on the ticks of this strength of dip were further confirmed by notes made on other bovines—not in experiment—which had been dipped at similar five-day intervals. The infestation of ticks on these animals was seen to increase, and, as in the case of .123 per cent. arsenical dip, complaints were made by the neighbouring farmers, whose cattle were dipped in the tank, stating that the ticks on their animals were not being killed. It may, therefore, be concluded that this strength of arsenic is not sufficient for the adequate destruction of ticks. The effect on the oxen in the latter stages of the experiment showed that, with oxen in fair condition and not doing too severe work, this strength could with safety be used even during very warm weather.

EXPERIMENT D.

A further test with dip containing an increased quantity of arsenic was now commenced, as the tick-killing effects of a .128 per cent. solution of arsenic proved unsatisfactory. The strength was .130 per cent. arsenic, and dipping of the animals was commenced on 9th October, 1914. It was anticipated that this experiment would yield some valuable information, as from previous experiments it was deduced that this strength of arsenic would give satisfactory results, and as the time of the year was most suitable for tick infestation, a good idea would be gained of the effect of a dip containing this percentage of arsenic on the ticks. The experiment was continued at the usual five-day dipping intervals until 28th November, 1914, during which time the animals received ten dippings.

Effect on Ticks of .130 per cent. Arsenic.—The lethal action of this dip containing .130 per cent. arsenic on ticks was unsatisfactory. Under the tail 52.3 per cent. and on the brush 14.5 per cent. were killed during the experiment, *vide* Table D (I). On comparing this with the results obtained with .128 per cent. arsenic, it will be seen that there is a considerable decrease in the number of ticks killed. For this it is difficult to offer a satisfactory explanation. In my opinion it was due to the fact that during this period the ticks have been considerably more active than formerly, reinfestation occurred more quickly, and many of the ticks which died and dropped off, between the times of observation, were replaced by freshly picked up ticks, and thus the percentage of killed ticks found was considerably reduced. This error in observation could not, however, be overcome while the experiments were being conducted under these conditions, as only ticks found killed and attached could be recorded, and ticks which died and dropped off escape observation.

From the point of view of practical dipping, this strength of dip must be regarded as unsatisfactory.

Effect on Oxen in Work.—In the "Dipped lot" no distress during work was observed until after the fifth dipping, 30th October, 1914. On that day all the "Dipped lot" showed slight increased respirations. The "Control lot" remained normal. From this date until the termination of the experiment the symptoms in the "Dipped lot" appeared to become more and more aggravated at each observation. Towards the end of the experiment the amount of work had to be reduced on account of the severity of the symptoms. Marked respiratory distress, salivation, hanging back in the yoke were all symptoms shown by the four animals constituting the "Dipped lot," while no symptoms of distress in excess of the normal were shown at any time by the "Control" animals.

General Observation on the Experiments.—Considerable variation was observed in the action of the various dips in these experiments depending on the percentage of arsenic present, climatic conditions, and the general condition of the animals. It was observed that, if a hot dry day followed the dipping, the lethal effect on the ticks was not nearly so great as when dipping took place in moist, humid weather. This was interpreted to be due to the fact that a solution of the dip was in contact with the tick for a much longer period in the latter case, and, as a result of this prolonged action, greater absorption by the ticks took place, resulting in increased mortality. The effect of the varying dips used on the brush was to a great extent interfered with by the amount of hair present, which prevented complete penetration. The time which an animal took to go through the dip varied from seven to ten seconds, and an examination of the tails afterwards showed that in some cases the skin of the tail in the brush and the attached ticks were quite dry. Taking these facts into consideration it is easy to understand the small percentage of ticks killed in the brush, as compared with those under the tail.

The length of this tank from the take-off to the top of the exit slope is 30 feet—giving a swim of about 20 feet.

The results obtained in the preceding experiments showed that a dipping fluid containing a higher percentage of arsenic than .128 per cent. could not be used on oxen at five-day intervals without seriously interfering with their working capacity, also that a dipping

fluid of this strength used in a tank giving only a 25-foot swim was not found to possess the tick-killing effect which was considered essential for practical purposes.

EXPERIMENT E.

The tank used in these previous experiments was erected in the early days of dipping, and the swim given is much less than that usually arranged for in modern dipping tanks. It was, therefore, decided to repeat the experiment with dipping fluid containing .128 per cent. arsenic with the addition of soap and paraffin, using a "walk-in" tank, giving a swim of about 35 feet, which is about the average length of swim arranged now for in dipping tanks. This further experiment was undertaken in December, 1914, and continued at five-day intervals until February, 1915, fourteen dippings being given in this period.

Effect on Animals in Work.—During the observation only on one occasion were symptoms shown—in the "Dipped lot"—of distress during work. This was on 14th December, 1914, viz., the second day after the second dipping. No further distress was noticed during subsequent observations.

Effect on Ticks.—The average percentage of ticks killed with each dipping was: under tail, 50.5 per cent.; in brush, 19.3 per cent. This small percentage in the latter case was undoubtedly due to insufficient penetration of the dip through the hair of the brush. [Charts E (I) to E (IV) refer.]

General observations on the dipped animals showed that ticks were present in the ears, under the tail, and in the brush, while the body was free from ticks. Further, that the development of the ticks in the two latter situations was seriously interfered with and that engorged females were very rarely seen, although they were commonly present in the "Control" animals. The following chart gives the comparative infestation present in the two lots of animals, the first tick count being made about the middle of the experiment, and the second a month later.

Tick Observations on Oxen Dipped with .128 per cent. Arsenic.

No. 1.

DATE.	No.	AREAS ON WHICH TICKS WERE PRESENT.						
		Ears.	Dewlap.	Perineum.	Neck.	Brush.	Under Tail.	
20.1.15	87	Numerous larvae, few adults	Nil	Nil	Nil	19	33	Dipped.
	88	Few.	"	"	"	6	33	
	89	"	"	"	"	3	3	
	90	"	"	"	"	9	31	
	91	Numerous	Numerous	Numerous	Frequent	1	50	Controls.
	92	"	"	"	Few nymphae	5	84	
	93	"	"	"	Frequent	17	64	
	94	"	"	"	Nil	10	53	

DATE.	No.	AREAS ON WHICH TICKS WERE PRESENT.				
		Ears.	Brisket.	Hind Legs.	Neck.	
15.2.15	87	Few larvae, few adults	—	—	—	Dipped lot.
	88	Few adults	—	—	—	
	89	Numerous larvae, adults rare	—	—	—	
	90	Adults rare	—	—	—	
	91	Adults and larvae frequent	Few	Few	Rare	Controls.
	92	"	Numerous	Numerous	"	
	93	Rare	"	"	Nymphae frequent	
	94	Larvae and nymphae numerous, few adults	"	"	Frequent	

It will be noted that these observations were made at a time of the year when ticks were most active, and the absence of ticks on the body of the dipped animals shows that the arsenic was present in sufficient quantity to kill any ticks with which it was able to come into immediate contact. In "walk-in" tanks the difficulty of controlling tick infestation of the ears and head is greater than in "plunge" tanks, and even though the head is pushed under the surface during the swim there is not the same amount of penetration of dipping fluid to all the recesses that one gets with the latter type of tank.

Conclusions.—The method of determining the percentage of ticks killed by counting the number present on successive days in the tail tuft and under the base of the tail is one which gives only an approximate estimate. General practical observations in the field show that ticks in these situations are almost impossible to eradicate with ordinary dipping methods, and farmers usually resort to hand-dressing of these areas as an essential additional means of control. The tails of the animals in the experiments were unclipped so as to assist as much as possible in the collection of ticks in this site, but incidentally this interfered with dip penetration and reduced the mortality among the ticks in this position in consequence. The mortality, therefore, represented in the tick counts shown in the tables is one which was considerably reduced owing to these unfavourable circumstances, and this fact must be borne in mind when going through the charts.

It will be seen from these foregoing experiments that, given a tank with a sufficiently long swim and dipping at five-day intervals it is possible to prevent tick infestation with a dipping fluid containing as much lower percentage than the standard .16 per cent., and that a dipping fluid containing .128 per cent. arsenic, while possessing sufficient poisonous properties to prevent tick infestation, is the maximum strength which can be used on oxen without producing a degree of respiratory distress incompatible with good work.

Table A (I).
LABORATORY DIP.
TICK KILLING OBSERVATION.

.123 per cent. Arsenical Solution with addition of Soft Soap and Paraffin.

Every 5 days.

Date.	Number of Dip-pings.	Total Number of Ticks Alive.	Brush.		Under Tail.		Total Killed.
			Alive.	Dead	Alive.	Dead.	
5.6.14.....	1	26	4	—	22	—	—
6.6.14.....			1	3	2	25	28
7.6.14.....			2	1	8	3	4
8.6.14.....			—	2	20	—	2
9.6.14.....			—	—	28	—	—
10.6.14.....	2	35	—	—	35	5	5
			Killed.....		—	33	39
11.6.14.....			—	—	19	27	27
12.6.14.....			—	—	15	16	16
13.6.14.....			—	—	23	2	2
14.6.14.....	3	33	—	—	22	7	7
15.6.14.....			—	—	33	—	—
			Killed.....		—	52	52
16.6.14.....			1	—	17	17	17
17.6.14.....			—	1	17	8	9
18.6.14.....	4	23	—	—	16	2	2
19.6.14.....			—	—	23	—	—
20.6.04.....			—	—	23	1	1
			Killed.....		1	28	29
21.6.14.....	5	25	—	—	13	16	16
22.6.14.....			—	—	9	7	7
23.6.14.....			—	—	23	—	—
24.6.14.....			—	—	22	2	2
25.6.14.....			—	—	25	3	3
	6	7	Killed.....		—	28	28
26.6.14.....			—	—	25	5	5
27.6.14.....			1	—	16	18	18
28.6.14.....			1	—	10	7	7
29.6.14.....			1	—	10	2	2
30.6.14.....			2	—	5	8	8
			Killed.....		—	40	40
1.7.14.....			2	—	7	4	4
2.7.14.....			2	—	7	1	1
3.7.14.....			2	—	11	—	—
4.7.14.....			2	—	31	1	1
5.7.14.....			2	—	27	6	6
			—	—	—	12	12

Table A (II).

CONTROLS.

Every 5 days.

Dipped in Water only.

Date.	Number of Dip- pings.	Total Number of Ticks Alive.	Brush.		Under Tail.		Total Dead.	Missing and En- gorged.
			Alive.	Dead.	Alive.	Dead.		
5.6.14....	1	109	4	—	105	—	—	—
6.6.14....			1	—	87	1	1	20
7.6.14....			1	—	82	6	6	1
8.6.14....			—	—	78	2	2	9
9.6.14....			—	—	81	—	—	4
10.6.14....	2	85	1	—	84	6	6	3
						15	15	37
11.6.14....	3	70	1	—	89	2	2	1
12.6.14....			1	—	87	1	1	6
13.6.14....			1	—	82	2	2	3
14.6.14....			1	—	78	2	2	2
15.6.14....			—	1	70	8	9	—
						15	16	12
16.6.14....	4	61	—	—	69	2	2	3
17.6.14....			1	—	68	2	2	4
18.6.14....			1	—	55	4	4	10
19.6.14....			1	—	55	1	1	—
20.6.14....			—	—	61	—	—	1
						9	9	18
21.6.14....	5	48	—	—	59	4	4	3
22.6.14....			—	—	60	2	2	—
23.6.14....			—	—	61	5	5	—
24.6.14....			—	—	53	2	2	6
25.6.14....			—	—	48	—	—	5
						13	13	14
26.6.14....	6	45	—	—	51	—	—	—
27.6.14....			—	—	46	—	—	5
28.6.14....			—	—	50	1	1	—
29.6.14....			—	—	42	3	3	7
30.6.14....			—	—	45	—	—	2
						4	4	14
1.7.14....			—	—	39	3	3	4
2.7.14....			—	—	39	—	—	2
3.7.14....			—	—	44	—	—	—
4.7.14....			—	—	48	1	1	—
5.7.14....			—	—	49	1	1	3
						5	5	9

Table A (III).

LABORATORY DIP.

TICK KILLING OBSERVATION.

0.123 per cent. Arsenic, with Soft Soap and Paraffin.

Every 5 days.

DATE.	EFFECT OF DIPPING. No.	BRUSH.				UNDER TAIL.			
		Number Initially Present.	Number which Attached.	Number Killed.	Percentage Killed.	Number Initially Present.	Number which Attached.	Number Killed.	Percentage Killed.
5.6.14 to 10.6.14.	1	4	2	6	100.0	22	46	33	48.9
11.6.14 to 15.6.14.	2	—	—	—	—	35	50	52	61.6
16.6.14 to 20.6.14.	3	—	1	1	100.0	33	18	28	54.9
21.6.14 to 25.6.14.	4	—	—	—	—	23	30	28	53.8
26.6.14 to 30.6.14.	5	—	2	—	—	25	20	40	88.8
1.7.14 to 5.7.14.	6	2	—	—	—	5	34	12	30.7
		Average					220	Average	56.3
		percentage						percentage	

Table A (V).

SUMMARY.

0.123 per cent. Arsenite.

	Date.	BRUSH.		UNDER TAIL.	
		Percentage of Ticks Killed.	Number of Ticks under Observation.	Percentage of Ticks Killed.	Number of Ticks under Observation.
Dipped lot....	5.6.14 to 5.7.14	50	9	56.3	220

	Date.	BRUSH.		UNDER TAIL.		BRUSH AND UNDER TAIL.	
		Percentage of Ticks Dead.	Number of Ticks under Observation.	Percentage of Ticks Dead.	Number of Ticks under Observation.	Percentage of Ticks Missing and Engorged.	Total Number of Ticks under Observation.
Control	5.6.14 to 5.7.14	33.3	6	12.2	184	20.1	190

Table B (I).

LABORATORY DIP.

TICK KILLING OBSERVATION.

0.133 per cent. Arsenical Solution, with addition of Soft Soap and Paraffin.

Dipped every 5 days.

Date.	Number of Dip-pings.	Total Number of Ticks Alive.	Brush.		Under Tail.		Total Killed.
			Alive.	Dead.	Alive.	Dead.	
5.7.14.....	1	29	2	—	27	—	—
6.7.14.....			1	1	8	19	20
7.7.14.....			3	—	5	12	12
8.7.14.....			3	—	9	3	3
9.7.14.....			3	—	31	1	1
10.7.14.....	2	37	3	—	34	1	1
			Total	Killed.		36	37
				1			
11.7.14.....	3	12	2	1	17	25	26
12.7.14.....			—	2	13	5	7
13.7.14.....			—	—	16	2	2
14.7.14.....			—	—	15	4	4
15.7.14.....			—	—	12	3	3
			Total	Killed.		39	42
				3			
16.7.14.....	4	8	—	—	5	7	7
17.7.14.....			1	—	3	2	2
18.7.14.....			1	—	2	1	1
19.7.14.....			2	—	5	—	—
20.7.14.....			3	—	5	—	—
			Total	Killed.		10	10
				—			
21.7.14.....	5	10	2	1	3	5	6
22.7.14.....			3	—	5	1	1
23.7.14.....			3	—	7	—	—
24.7.14.....			1	2	8	—	2
25.7.14.....			1	—	9	1	1
			Total	Killed.		7	10
				3			
26.7.14.....	6	40	3	2	9	4	6
27.7.14.....			3	—	40	13	13
28.7.14.....			3	—	40	2	2
29.7.14.....			6	—	33	7	7
30.7.14.....			6	—	34	5	5
			Total	Killed.		31	33
				2			
31.7.14.....			6	1	21	17	18
1.8.14.....			5	1	18	11	12
2.8.14.....			5	1	19	5	6
3.8.14.....			1	5	30	2	7
			Total	Killed.		35	43
				8			

Table B (II).

CONTROLS.

Every 5 days.

Dipped in Water only.

Date.	Number of Dip- pings.	Total Number of Ticks Alive.	Brush.		Under Tail.		Total Dead.	Missing and En- gorged.
			Alive.	Dead.	Alive.	Dead.		
5.7.14.....	1	19	—	—	49	—	—	—
6.7.14.....			—	—	52	2	2	—
7.7.14.....			—	—	55	—	—	4
8.7.14.....			—	—	54	2	2	1
9.7.14.....			—	—	51	—	—	3
10.7.14.....	2	47	3	—	44	3	3	4
				—		7	7	12
11.7.14.....			4	—	46	2	2	—
12.7.14.....			4	—	43	2	2	3
13.7.14.....			5	—	42	—	—	1
14.7.14.....	3	42	5	—	42	—	—	4
15.7.14.....			5	—	37	2	2	4
				—		6	6	12
16.7.14.....			3	1	31	1	2	6
17.7.14.....			3	—	33	1	1	—
18.7.14.....	4	31	3	—	30	—	—	3
19.7.14.....			4	—	30	1	1	—
20.7.14.....			3	1	28	2	3	1
				2		5	7	10
21.7.14.....			2	1	28	—	1	—
22.7.14.....	5	33	5	1	32	—	1	—
23.7.14.....			3	—	30	1	1	4
24.7.14.....			4	—	30	—	—	—
25.7.14.....			7	1	26	—	1	1
				3		1	4	5
26.7.14.....	6	65	5	—	28	1	1	—
27.7.14.....			5	—	36	3	3	1
28.7.14.....			13	—	41	—	—	—
29.7.14.....			18	—	44	1	1	1
30.7.14.....			19	—	46	—	—	1
			—		5	5	3	
31.7.14.....			14	2	53	2	14	5
1.8.14.....			15	—	40	1	1	13
2.8.14.....			15	—	36	1	1	3
3.8.14.....			16	—	34	1	1	5
					2		5	7

Table B (III).

LABORATORY DIP.

TICK KILLING OBSERVATION.

0.133 per cent. Arsenic, with Soft Soap and Paraffin.

Every 5 days.

DATE.	EFFECT OF DIPPING. No.	BRUSH.			UNDER TAIL.			
		Number Initially Present.	Number which Attached.	Number Killed.	Percentage Killed.	Number Initially Present.	Number which Attached.	Percentage Killed.
5.7.14 to 10.7.14..	1	2	2	1	25.0	27	36	57.1
11.7.14 to 15.7.14..	2	3	—	3	100.0	34	17	76.4
16.7.14 to 20.7.14..	3	—	3	—	0.0	12	5	57.8
21.7.14 to 25.7.14..	4	3	1	3	75.0	5	10	66.6
26.7.14 to 30.7.14..	5	1	7	2	25.0	9	56	47.3
31.7.14 to 3.8.14..	6	6	3	8	88.8	34	31	53.8
		Average percentage			37.3	Average percentage		
		18			37.3	182		
						61.5		

Table B (IV).

CONTROL

to

0.133 per cent. Arsenite.

Every 5 days.

DATE.	EFFECT OF DIPPING.	BRUSH.				UNDER TAIL.			UNDER TAIL AND BRUSH COMBINED.		
		Number Initially Present.	Number which Attached.	Number Dead.	Percent- age Dead.	Number Initially Present.	Number which Attached.	Number Dead.	Percent- age Dead.	Number of Missing and Engorged.	Percent- age of Missing and Engorged.
	No.										
5.7.14 to 10.7.14..	1	—	3	—	—	49	7	7	12.5	12	20.3
11.7.14 to 15.7.14..	2	3	2	—	—	44	4	6	12.5	12	22.6
16.7.14 to 20.7.14..	3	5	1	2	33.3	37	4	5	12.1	10	21.2
21.7.14 to 25.7.14..	4	3	9	3	25.0	28	4	1	3.1	5	11.3
26.7.14 to 30.7.14..	5	7	14	—	—	26	25	5	9.8	3	4.1
31.7.14 to 3.8.14..	6	19	2	2	9.5	46	9	5	9.0	26	34.2
				Average percent- age				Average percent- age		Average percent- age	
			31		11.3		102		9.8		18.77

Table B (V).

SUMMARY.

0.133 per cent. Arsenite.

	Date.	BRUSH.		UNDER TAIL.	
		Percentage of Ticks Killed.	Number of Ticks under Observation.	Percentage of Ticks Killed.	Number of Ticks under Observation.
Dipped lot....	5.7.14 to 3.8.14	37.3	18	61.5	182

	Date.	BRUSH.		UNDER TAIL.		BRUSH AND UNDER TAIL.	
		Percentage of Ticks Dead.	Number of Ticks under Observation.	Percentage of Ticks Dead.	Number of Ticks under Observation.	Percentage of Ticks Missing and Engorged.	Total Number of Ticks under Observation.
Controls	5.7.14 to 3.8.14	11.3	31	9.8	102	18.7	133

Table C (I).
LABORATORY DIP.

TICK KILLING OBSERVATION.

0.128 per cent. Arsenical Solution with addition of Soft Soap and Paraffin.
Every 5 days.

Date.	Number of Dip-pings.	Total Number of Ticks Alive.	Brush.		Under Tail.		Total Killed.
			Alive.	Dead.	Alive.	Dead.	
4.8.14.....	1	31	1	—	30	—	—
5.8.14.....			2	—	17	15	15
6.8.14.....			3	1	10	11	12
7.8.14.....			5	—	9	2	2
8.8.14.....			7	—	10	1	1
9.8.14.....	2	20	6	1	14	1	2
			Total	Killed.		30	32
10.8.14.....	3	60	6	2	38	10	12
11.8.14.....			4	2	36	14	16
12.8.14.....			4	—	31	12	12
13.8.14.....			2	2	50	2	4
14.8.14.....			3	—	57	—	—
			Total	Killed.		38	44
	4	61	3	—	49	17	17
15.8.14.....			2	1	40	27	28
16.8.14.....			4	1	53	9	10
17.8.14.....			1	—	50	9	9
18.8.14.....			1	—	58	5	5
19.8.14.....			2	1	59	5	6
20.8.14.....			Total	Killed.		72	75
21.8.14.....	5	54	1	1	32	41	42
22.8.14.....			1	—	26	12	12
23.8.14.....			1	—	36	8	8
24.8.14.....			8	—	39	9	9
25.8.14.....			7	1	47	2	3
			Total	Killed.		72	74
	6	131	8	2	13	38	40
26.8.14.....			38	2	9	13	15
27.8.14.....			54	2	28	3	5
28.8.14.....			51	3	49	5	8
29.8.14.....			59	1	72	2	3
30.8.14.....			Total	Killed.		61	71
31.8.14.....	7	185	64	3	79	29	32
1.9.14.....			63	4	86	23	25
2.9.14.....			47	7	95	19	26
3.9.14.....			56	1	100	16	17
4.9.14.....			70	3	115	8	11
			Total	Killed.		95	113
	8	131	60	12	67	77	89
5.9.14.....			56	5	27	55	60
6.9.14.....			65	4	35	14	18
7.9.14.....			66	3	55	10	13
8.9.14.....			66	5	65	6	11
9.9.14.....			Total	Killed.		162	191

Table C (I)—(continued).

LABORATORY DIP.

TICK KILLING OBSERVATION.

0.128 per cent. Arsenical Solution with addition of Soft Soap and Paraffin.
Every 5 days.

Date.	Number of Dip- pings.	Total Number of Ticks Alive.	Brush.		Under Tail.		Total Killed.
			Alive.	Dead.	Alive.	Dead.	
10.9.14.....	9	172	66	6	46	37	43
11.9.14.....			71	3	30	23	26
12.9.14.....			65	5	42	5	10
13.9.14.....			70	5	59	4	9
14.9.14.....			74	4	98	3	7
		Total	Killed.	23		72	95
15.9.14.....	10	227	71	13	59	59	72
16.9.14.....			68	6	20	51	57
17.9.14.....			80	4	25	10	14
18.9.14.....			90	4	34	5	9
19.9.14.....			160	—	67	1	1
		Total	Killed.	27		126	153
20.9.14.....	11	204	158	7	84	24	31
21.9.14.....			163	13	65	28	41
22.9.14.....			160	6	52	15	21
23.9.14.....			150	4	66	2	6
24.9.14.....			125	3	79	2	5
		Total	Killed.	33		71	104
25.9.14.....	12	159	102	15	48	40	55
26.9.14.....			99	4	65	11	15
27.9.14.....			73	12	73	10	22
28.9.14.....			65	3	77	7	10
29.9.14.....			70	1	89	3	4
		Total	Killed.	35		71	106
30.9.14.....	13	122	61	9	64	32	41
1.10.14.....			67	2	52	24	26
2.10.14.....			65	2	57	14	16
3.10.14.....			82	6	41	12	18
4.10.14.....			77	1	45	2	3
		Total	Killed.	20		84	104
5.10.14.....			83	4	30	19	23
6.10.14.....			93	—	32	13	13
7.10.14.....			113	1	35	6	7
8.10.14.....			112	1	33	8	9
9.10.14.....			132	—	39	5	5
		Total	Killed.	6		51	57

Experiment with .128 per cent. Arsenical Solution concluded.

Table C (II).

CONTROLS.

Every 5 days.

Date.	Number of Dip- pings.	Total Number of Ticks Alive.	Brush.		Under Tail.		Total Dead.	Missing and En- gorged.
			Alive.	Dead.	Alive.	Dead.		
4.8.14.....	1	67	17	—	50	—	—	—
5.8.14.....			18	—	47	1	1	5
6.8.14.....			19	—	49	2	2	4
7.8.14.....			17	2	47	—	2	3
8.8.14.....			18	—	49	—	—	—
9.8.14.....	2	62	19	—	43	1	1	7
				2		4	6	19
10.8.14.....			15	—	51	—	—	4
11.8.14.....			15	—	62	—	—	—
12.8.14.....			20	—	64	1	1	2
13.8.14.....	3	87	15	4	73	—	4	3
14.8.14.....			15	1	72	1	2	2
				5		2	7	11
15.8.14.....			18	—	77	—	—	—
16.8.14.....			20	1	64	1	2	9
17.8.14.....	4	80	18	—	69	1	1	8
18.8.14.....			7	—	61	—	—	21
19.8.14.....			16	—	63	—	—	1
20.8.14.....			15	—	65	—	—	3
				1		2	3	42
21.8.14.....	5	83	13	1	61	3	4	4
22.8.14.....			13	1	64	—	1	3
23.8.14.....			12	—	65	1	1	3
24.8.14.....			14	—	71	—	—	—
25.8.14.....			13	—	70	1	1	2
	6	114		2		5	7	12
26.8.14.....			14	—	69	2	2	2
27.8.14.....			31	—	70	1	1	2
28.8.14.....			35	1	79	—	1	2
29.8.14.....			33	1	86	—	1	4
30.8.14.....	7	121	31	—	83	—	—	16
				2		3	5	20
31.8.14.....			37	—	82	1	1	2
1.9.14.....			40	1	85	—	1	3
2.9.14.....			37	—	89	1	1	3
3.9.14.....	8	98	34	—	106	1	1	3
4.9.14.....			29	—	92	1	1	20
				1		4	5	31
5.9.14.....			28	1	98	—	1	—
6.9.14.....			28	—	82	—	—	16
7.9.14.....	9	98	28	—	73	2	2	11
8.9.14.....			38	—	83	—	—	—
9.9.14.....			34	1	64	2	3	21
				2		4	6	48

Table C (II)—(continued).

CONTROLS.

Every 5 days.

Date.	Number of Dip-pings.	Total Number of Ticks Alive.	Brush.		Under Tail.		Total Dead.	Missing and En-gorged.
			Alive.	Dead.	Alive.	Dead.		
9.9.14.....	8	98	34	—	64	—	—	—
10.9.14.....			27	—	63	—	—	8
11.9.14.....			26	1	64	1	2	2
12.9.14.....			25	—	68	—	—	8
13.9.14.....			28	—	71	1	1	4
14.9.14.....	9	109	31	—	78	—	—	3
				1		2	3	25
15.9.14.....			37	—	84	1	1	2
16.9.14.....			53	1	67	2	3	15
17.9.14.....			46	4	71	—	4	5
18.9.14.....	10	170	45	—	79	1	1	1
19.9.14.....			103	1	67	1	2	11
				6		5	11	34
20.9.14.....			104	1	60	—	1	5
21.9.14.....			95	5	81	1	6	6
22.9.14.....	11	148	85	1	70	2	3	19
23.9.14.....			82	1	69	1	2	8
24.9.14.....			70	1	78	—	1	11
				9		4	13	49
25.9.14.....			81	—	86	—	—	2
26.9.14.....	12	160	71	2	100	—	2	101
27.9.14.....			72	—	99	—	—	16
28.9.14.....			55	2	104	1	3	17
29.9.14.....			65	2	95	1	3	7
				6		2	8	53
30.9.14....	13	157	69	1	94	2	3	10
1.10.14....			71	1	92	2	3	7
2.10.14....			80	—	86	—	—	8
3.10.14....			85	1	80	1	2	19
4.10.14....			78	1	79	1	2	16
				4		6	10	60
5.10.14....			71	—	71	—	—	20
6.10.14....			64	1	77	—	1	13
7.10.14....			76	—	71	1	1	13
8.10.14....			69	1	71	1	2	13
9.10.14....			76	—	87	—	—	3
				2		2	4	62

Experiment with .128 per cent. Arsenic Solution concluded.

Table C (III).

LABORATORY DIP.

TICK KILLING OBSERVATION.

Every 5 days. 0.128 per cent. Arsenic, with Soft Soap and Paraffin.

DATE.	EFFECT OF DIPPING.	BRUSH.				UNDER TAIL.			
		No.	Number Initially Present.	Number which Attached.	Number Killed.	Percentage Killed.	Number Initially Present.	Number which Attached.	Number Killed.
4. 8. 14 to 9. 8. 14	1	1	7	2	25.0	30	14	30	68.1
10. 8. 14 to 14. 8. 14	2	6	3	6	66.6	14	81	38	40.0
15. 8. 14 to 20. 8. 14	3	3	5	3	37.5	57	74	72	54.9
21. 8. 14 to 25. 8. 14	4	2	7	2	22.2	59	60	72	60.5
26. 8. 14 to 30. 8. 14	5	7	62	10	14.4	47	86	61	45.8
31. 8. 14 to 4. 8. 14	6	59	38	18	18.5	72	138	95	45.2
5. 9. 14 to 9. 9. 14	7	70	25	29	30.5	115	112	162	71.3
10. 9. 14 to 14. 9. 14	8	66	32	23	23.4	65	105	72	68.7
15. 9. 14 to 19. 9. 14	9	74	113	27	23.8	98	95	126	65.2
20. 9. 14 to 24. 9. 14	10	160	26	33	17.7	67	83	71	47.3
25. 9. 14 to 29. 9. 14	11	125	7	35	26.5	79	81	71	44.3
30. 9. 14 to 4. 10. 14	12	70	31	20	19.8	89	44	84	63.1
5. 10. 14 to 9. 10. 14	13	77	51	6	4.6	45	45	51	56.6
			Average	percentage	25.4		1048	Average	percentage
			408						56.2

Table C (IV).

CONTROL

to

0.128 per cent. Arsenite.

DATE.	EFFECT OF DIPPING. No.	BRUSH.			UNDER TAIL.				UNDER TAIL AND BRUSH COMBINED		
		Number Initially Present.	Number which Attached.	Number Dead.	Percent- age Dead.	Number Initially Present.	Number which Attached.	Number Dead.	Percent- age Dead.	Number of Missing and Engorged.	Percent- age of Missing and Engorged.
4. 8.14 to 9. 8.14	1	17	4	2	9.5	50	6	4	7.1	19	24.6
10. 8.14 to 14. 8.14	2	19	6	5	20.6	43	31	2	2.7	11	11.1
15. 8.14 to 20. 8.14	3	15	15	1	3.3	72	15	2	2.3	42	35.8
21. 8.14 to 25. 8.14	4	15	2	2	11.7	65	8	5	6.8	12	13.3
26. 8.14 to 30. 8.14	5	13	23	2	5.5	70	19	3	3.3	20	16.0
31. 8.14 to 4. 9.14	6	31	10	1	2.4	83	26	4	3.6	31	20.6
5. 9.14 to 9. 9.14	7	29	10	2	5.1	92	16	4	3.6	48	3.2
10. 9.14 to 14. 9.14	8	34	6	1	2.5	64	17	2	2.4	25	20.6
15. 9.14 to 19. 9.14	9	31	82	6	5.3	78	19	5	5.1	34	22.6
20. 9.14 to 24. 9.14	10	103	2	9	8.5	67	31	4	4.0	49	24.1
25. 9.14 to 29. 9.14	11	70	23	6	6.4	78	28	2	1.8	53	26.6
30. 9.14 to 4.10.14	12	65	23	4	4.5	95	1	6	6.2	60	32.6
5.10.14 to 9.10.14	13	78	19	2	2.0	79	22	2	1.9	62	31.3
				Average percent- age				Average percent- age		Average percent- age	
			242		6.6		289		3.9		21.7

Table C (V).

SUMMARY.

0.128 per cent. Arsenite.

	Date.	BRUSH.		UNDER TAIL.	
		Percentage of Ticks Killed.	Number of Ticks under Observation.	Percentage of Ticks Killed.	Number of Ticks under Observation.
Dipped lot....	4.8.14 to 9.10.14	25.4	408	56.2	1048

	Date.	BRUSH.		UNDER TAIL.		BRUSH AND UNDER TAIL.	
		Percentage of Ticks Dead.	Number of Ticks under Observation.	Percentage of Ticks Dead.	Number of Ticks under Observation.	Percentage of Ticks Missing and Engorged.	Total Number of Ticks under Observation.
Controls	4.8.14 to 9.10.14	6.6	242	3.9	289	21.7	531

Table D (I).

.130 per cent. Arsenical Solution.

LABORATORY DIP.

TICK KILLING OBSERVATION.

Every 5 days.

Date.	Number of Dip-pings.	Total Number of Ticks Alive.	Brush.		Under Tail.		Total Killed.
			Alive.	Dead.	Alive.	Dead.	
9.10.14.....	1	171	132	—	39	—	—
10.10.14.....			107	8	41	21	29
11.10.14.....			135	11	29	26	37
12.10.14.....			125	1	64	6	7
13.10.14.....			156	3	69	6	9
14.10.14.....	2	223	151	2	72	5	7
			Total	Killed.		64	89
				25			
15.10.14.....	3	117	132	8	11	73	81
16.10.14.....			108	6	7	7	13
17.10.14.....			112	2	20	—	2
18.10.14.....			104	3	22	3	6
19.10.14.....			89	1	28	2	3
			Total	Killed.		85	105
				20			
20.10.14.....	4	103	84	—	10	18	18
21.10.14.....			80	5	8	7	12
22.10.14.....			81	5	12	3	8
23.10.14.....			79	1	19	—	1
24.10.14.....			83	—	20	1	1
			Total	Killed.		29	40
				11			
25.10.14.....	5	141	79	3	18	8	11
26.10.14.....			88	1	37	5	6
27.10.14.....			71	6	40	3	9
28.10.14.....			73	2	41	3	5
29.10.14.....			91	4	50	—	4
			Total	Killed.		19	35
				16			
30.10.14.....	6	124	80	11	36	17	28
31.10.14.....			73	4	34	10	14
1.11.14.....			60	2	28	12	14
2.11.14.....			87	—	53	6	6
3.11.14.....			67	—	57	1	1
			Total	Killed.		46	63
				17			
4.11.14.....	7	134	78	6	34	32	38
5.11.14.....			88	5	26	14	19
6.11.14.....			101	3	38	4	7
7.11.14.....			97	1	39	5	6
8.11.14.....			85	—	49	1	1
			Total	Killed.		56	71
				15			

Table D (I)—(continued).

.130 per cent. Arsenical Solution.

LABORATORY DIP.

TICK KILLING OBSERVATION.

Every 5 days.

Date.	Number of Dip-pings.	Total Number of Ticks Alive.	Brush.		Under Tail.		Total Killed.
			Alive.	Dead.	Alive.	Dead.	
9.11.14.....	8	141	81	7	34	36	43
10.11.14.....			79	2	27	16	18
11.11.14.....			80	1	39	3	4
12.11.14.....			85	1	47	4	5
13.11.14.....			96	1	45	1	2
		Total	Killed.	12		60	72
14.11.14.....	9	118	90	7	32	16	23
15.11.14.....			70	6	33	19	25
16.11.14.....			65	1	41	12	13
17.11.14.....			61	1	60	2	3
18.11.14.....			68	1	50	17	18
		Total	Killed.	16		66	82
19.11.14.....	10	102	76	4	18	35	39
20.11.14.....			88	3	17	8	11
21.11.14.....			105	2	22	3	5
22.11.14.....			84	12	21	3	15
23.11.14.....			73	1	29	2	3
		Total	Killed.	22		51	73
24.11.14.....	11	128	72	10	28	13	23
25.11.14.....			45	5	27	12	17
26.11.14.....			60	7	30	3	10
27.11.14.....			70	1	45	2	3
28.11.14.....			62	2	66	—	2
		Total	Killed.	25		30	55

Table D (II).

CONTROLS.

Every 5 days.

Date.	Number of Dip-pings.	Total Number of Ticks Alive.	Brush.		Under Tail.		Total Dead.	Missing and En-gorged.
			Alive.	Dead.	Alive.	Dead.		
9.10.14....	1	163	76	—	87	—	—	—
10.10.14....			66	1	72	1	2	23
11.10.14....			57	5	68	3	8	16
12.10.14....			51	—	77	—	—	16
13.10.14....			87	—	92	—	—	12
14.10.14....			81	—	93	1	1	9
	2	174		6		5	11	76
15.10.14....			59	4	92	—	4	32
16.10.14....			52	—	81	—	—	23
17.10.14....			50	—	80	—	—	12
18.10.14....			47	—	90	4	4	9
19.10.14....			44	—	94	—	—	13
	3	138		4		4	8	89
20.10.14....			63	1	78	—	1	3
21.10.14....			59	1	74	—	1	12
22.10.14....			59	5	79	—	5	3
23.10.14....			64	—	84	—	—	2
24.10.14....			72	—	78	—	—	10
	4	150		7		—	7	30
25.10.14....			82	—	67	3	3	16
26.10.14....			58	1	99	—	1	26
27.10.14....			63	1	108	1	2	4
28.10.14....			59	1	97	3	4	12
29.10.14....			62	—	85	—	—	22
	5	147		3		7	10	80
30.10.14....			56	1	77	—	1	31
31.10.14....			49	3	92	1	4	4
1.11.14....			57	—	92	—	—	14
2.11.14....			55	—	101	1	1	10
3.11.14....			55	—	104	—	—	9
	6	159		4		2	6	68
4.11.14....			51	—	81	—	—	22
5.11.14....			75	1	94	—	1	5
6.11.14....			77	1	94	—	1	4
7.11.14....			83	—	101	—	—	3
8.11.14....			70	—	101	1	1	19
	7	171		2		1	3	53

Table D (II)—(continued).

CONTROLS.

Every 5 days.

Date.	Number of Dip-pings.	Total Number of Ticks Alive.	Brush.		Under Tail.		Total Dead.	Missing and En-gorged.
			Alive.	Dead.	Alive.	Dead.		
9.11.14....	8	197	75	1	105	1	2	6
10.11.14....			86	1	83	—	1	32
11.11.14....			90	—	103	—	—	8
12.11.14....			84	—	81	—	—	36
13.11.14....			87	—	110	—	—	4
				2		1	3	86
14.11.14....	9	174	83	1	90	—	1	38
15.11.14....			83	1	88	—	1	13
16.11.14....			81	—	99	—	—	2
17.11.14....			71	—	115	—	—	33
18.11.14....			65	—	109	—	—	26
				2		—	2	112
19.11.14....	10	164	67	—	101	—	—	12
20.11.14....			55	—	106	1	1	21
21.11.14....			76	—	96	1	1	12
22.11.14....			62	1	106	—	1	6
23.11.14....			67	—	97	—	—	12
				1		2	3	63
24.11.14....	11		56	—	92	—	—	18
25.11.14....			57	1	98	—	1	8
26.11.14....			44	—	95	—	—	21
27.11.14....			43	—	86	—	—	17
28.11.14....			45	—	120	—	—	10
				1		—	1	74

Table D (III).

LABORATORY DIP.

TICK KILLING OBSERVATION.

Every 5 days. 0.130 per cent. Arsenic, with Soft Soap and Paraffin.

DATE.	EFFECT OF DIPPING.	BRUSH.				UNDER TAIL.				
		No.	Number Initially Present.	Number which Attached.	Number Killed.	Percentage Killed.	Number Initially Present.	Number which Attached.	Number Killed.	Percentage Killed.
9. 10. 14 to 14. 10. 14		1	132	64	25	12.2	39	97	64	47.0
15. 10. 14 to 19. 10. 14		2	151	6	20	12.7	72	38	85	77.2
20. 10. 14 to 24. 10. 14		3	89	11	11	12.0	28	16	29	65.9
25. 10. 14 to 29. 10. 14		4	83	36	16	13.4	20	49	19	27.5
30. 10. 14 to 3. 11. 14		5	91	27	17	14.4	50	53	46	44.6
4. 11. 14 to 8. 11. 14		6	67	48	15	13.0	57	48	56	53.3
9. 11. 14 to 13. 11. 14		7	85	21	12	11.3	49	57	60	56.6
14. 11. 14 to 18. 11. 14		8	96	9	16	15.2	45	71	66	56.8
19. 11. 14 to 23. 11. 14		9	68	46	22	19.2	50	30	51	63.7
24. 11. 14 to 28. 11. 14		10	73	42	25	21.7	29	67	30	31.2
			Number of Ticks under Observation	442	Average percentage	14.5	Number of Ticks under Observation	565	Average percentage	52.3

Table D (IV).

CONTROL

to

0.130 per cent. Arsenic.

Every 5 days.

DATE.	EFFECT OF DIPPING.	BRUSH.				UNDER TAIL.				UNDER TAIL AND BRUSH COMBINED.	
		Number Initially Present.	Number which Attached.	Number Dead.	Percent- age Dead.	Number Initially Present.	Number which Attached.	Number Dead.	Percent- age Dead.	Number of Missing and Engorged.	Percent- age of Missing and Engorged.
9. 10. 14 to 14. 10. 14	1	163	36	6	3.0	87	26	5	4.4	76	24.3
15. 10. 14 to 19. 10. 14	2	81	—	4	4.9	93	14	4	3.7	89	47.3
20. 10. 14 to 24. 10. 14	3	44	38	7	8.5	94	10	—	—	30	16.1
25. 10. 14 to 29. 10. 14	4	72	19	3	3.2	78	42	7	5.8	80	37.9
30. 10. 14 to 3. 11. 14	5	62	8	4	5.7	85	29	2	1.7	68	36.9
4. 11. 14 to 8. 11. 14	6	55	34	2	2.2	104	21	1	0.8	53	24.7
9. 11. 14 to 13. 11. 14	7	70	25	2	2.1	101	54	1	0.6	86	34.4
14. 11. 14 to 18. 11. 14	8	87	—	2	2.3	110	27	—	—	112	50.0
19. 11. 14 to 23. 11. 14	9	65	28	1	1.0	109	16	2	1.6	63	24.3
24. 11. 14 to 28. 11. 14	10	67	4	1	1.4	97	34	—	—	74	36.6
		No. of Ticks under Observa- tion	355	Average percent- age	3.4	No. of Ticks under Observa- tion	360	Average percent- age	1.8	Average percent- age	33.2

Table D (V).

SUMMARY.

0.130 per cent. Arsenite.

Date.	BRUSH.		UNDER TAIL.	
	Percentage of Ticks Killed.	Number of Ticks under Obser- vation.	Percentage of Ticks Killed.	Number of Ticks under Obser- vation.
Dipped lot.... 9.10.14 to 28.11.14	14.5	442	52.3	565

Date.	BRUSH.		UNDER TAIL.		BRUSH AND UNDER TAIL.	
	Percent- age of Ticks Dead.	Number of Ticks under Observa- tion.	Percent- age of Ticks Dead.	Number of Ticks under Observa- tion.	Percent- age of Ticks Missing and Engorged.	Total Number of Ticks under Observa- tion.
Controls 9.10.14 to 28.11.14	3.4	355	1.8	360	33.2	715

Table E (I).
LABORATORY DIP.

TICK KILLING OBSERVATION.

0.128 per cent. Arsenical Solution with addition of Soft Soap and Paraffin.
Every 5 days.

Date.	Number of Dip- pings.	Total Number of Ticks Alive.	Brush.		Under Tail.		Total Killed.
			Alive.	Dead.	Alive.	Dead.	
7.12.14.....	1	95	38	-	57	-	-
8.12.14.....			50	2	69	14	16
9.12.14.....			48	1	54	16	17
10.12.14.....			36	-	37	11	11
11.12.14.....			47	1	42	5	6
12.12.14.....	2	109	59	-	50	5	5
			Killed.....			51	55
13.12.14.....			49	10	35	23	33
14.12.14.....			45	4	30	22	26
15.12.14.....			41	2	41	4	6
16.12.14.....			30	2	39	7	9
17.12.14.....	3	80	30	3	50	3	5
			Killed.....			58	79
18.12.14.....			28	4	30	29	33
19.12.14.....			66	1	56	9	10
20.12.14.....			61	-	59	3	3
21.12.14.....			49	-	75	3	3
22.12.14.....	4	180	92	-	88	-	1
			Killed.....			44	49
23.12.14.....			72	5	50	43	48
24.12.14.....			80	7	39	22	29
25.12.14.....			55	4	58	4	8
26.12.14.....			43	10	67	5	15
27.12.14.....	5	100	38	6	62	11	17
			Killed.....			85	117
28.12.14.....			44	3	98	16	19
29.12.14.....			42	5	93	26	31
30.12.14.....			50	4	106	11	15
31.12.14.....			49	3	115	2	5
1. 1.15.....	6	182	67	-	115	9	9
			Killed.....			64	79
2. 1.15.....			62	8	110	35	43
3. 1.15.....			77	2	92	52	54
4. 1.15.....			79	3	178	14	17
5. 1.15.....			61	-	189	7	7
6. 1.15.....	7	258	63	2	195	-	2
			Killed.....			108	123
7. 1.15.....			66	9	39	151	160
8. 1.15.....			63	4	45	17	21
9. 1.15.....			50	8	57	13	21
10. 1.15.....			45	1	60	7	8
11. 1.15.....	8	174	47	1	127	-	1
			Killed.....			188	211

Table E (I)—(continued).

LABORATORY DIP.

TICK KILLING OBSERVATION.

0.128 per cent. Arsenical Solution with addition of Soft Soap and Paraffin.

Every 5 days.

Date.	Number of Dip- pings.	Total Number of Ticks Alive.	Brush.		Under Tail.		Total Killed.
			Alive.	Dead.	Alive.	Dead.	
12. 1.15.....	9	128	34	14	74	61	75
13. 1.15.....			39	4	56	28	32
14. 1.15.....			52	—	66	16	16
15. 1.15.....			48	3	56	21	24
16. 1.15.....			56	3	72	2	5
			Killed.....			128	152
17. 1.15.....	10	184	52	4	37	47	51
18. 1.15.....			33	5	71	19	24
19. 1.15.....			33	1	93	7	8
20. 1.15.....			37	—	120	4	4
21. 1.15.....			40	1	144	4	5
			Killed.....			81	92
22. 1.15.....	11	207	31	4	91	69	73
23. 1.15.....			58	10	73	45	55
24. 1.15.....			62	2	70	31	33
25. 1.15.....			72	—	95	12	12
26. 1.15.....			79	1	128	—	1
			Killed.....			157	174
27. 1.15.....	12	161	71	12	64	64	76
28. 1.15.....			91	5	71	41	46
29. 1.15.....			91	1	49	22	23
30. 1.15.....			95	4	51	12	16
31. 1.15.....			88	3	73	1	4
			Killed.....			140	165
1. 2.15.....	13	138	70	8	43	34	42
2. 2.15.....			69	1	68	13	14
3. 2.15.....			56	—	51	22	22
4. 2.15.....			61	1	63	9	10
5. 2.15.....			67	2	71	4	6
			Killed.....			82	94
6. 2.15.....	14	183	52	14	40	37	51
7. 2.15.....			68	1	40	22	23
8. 2.15.....			75	7	62	13	20
9. 2.15.....			66	7	85	14	21
10. 2.15.....			87	5	96	6	11
			Killed.....			92	126
11. 2.15.....			66	8	63	40	48
12. 2.15.....			68	5	43	30	35
13. 2.15.....			79	—	57	20	20
14. 2.15.....			73	—	59	2	2
15. 2.15.....			65	2	59	4	6
			Killed.....			96	111

Table E (II).

CONTROLS.

Dipped in Water only.

Every 5 days.

Date.	Number of Dip- pings.	Total Number of Ticks Alive.	Brush.		Under Tail.		Total Dead.	Missing and En- gorged.
			Alive.	Dead.	Alive.	Dead.		
7.12.14....	1	105	36	—	79	—	—	—
8.12.14....			34	—	143	—	—	8
9.12.14....			49	—	135	—	—	10
10.12.14....			31	1	149	—	1	17
11.12.14....			34	—	136	—	—	16
12.12.14....	2	174	40	—	134	—	—	6
				1			1	57
13.12.14....			42	4	131	—	4	9
14.12.14....			29	2	135	—	2	16
15.12.14....			29	—	129	—	—	12
16.12.14....			22	—	127	—	—	16
17.12.14....	3	159	32	—	127	—	—	9
				6		—	6	62
18.12.14....			35	1	133	1	2	7
19.12.14....			56	—	157	1	1	—
20.12.14....			64	—	154	3	3	—
21.12.14....			47	—	169	—	—	17
22.12.14....	4	228	62	—	166	—	—	3
				1		5	6	27
23.12.14....			65	—	142	—	—	40
24.12.14....			40	—	130	—	—	46
25.12.14....			43	—	102	—	—	18
26.12.14....			21	—	111	2	2	27
27.12.14....	5	191	35	—	156	—	—	4
				—		2	2	135
28.12.14....			41	—	165	—	—	11
29.12.14....			52	1	164	2	3	8
30.12.14....			56	2	176	—	2	11
31.12.14....			57	—	157	—	—	29
1. 1.15....	6	224	54	—	170	2	2	14
				3		4	7	73
2. 1.15....			47	1	170	1	2	22
3. 1.15....			40	—	176	—	—	6
4. 1.15....			40	—	214	—	—	5
5. 1.15....			48	—	223	—	—	9
6. 1.15....	7	267	55	—	212	—	—	11
				1		1	2	53
7. 1.15....			48	1	216	—	1	13
8. 1.15....			47	—	221	—	—	15
9. 1.15....			40	—	194	—	—	37
10. 1.15....			35	—	169	—	—	30
11. 1.15....	8	253	36	—	217	—	—	10
				1		—	1	105

Table E (II)—(continued).

CONTROLS.

Dipped in Water only.

Every 5 days.

Date.	Number of Dip- pings.	Total Number of Ticks Alive.	Brush.		Under Tail.		Total Dead.	Missing and En- gorged.
			Alive.	Dead.	Alive.	Dead.		
12. 1.15....			44	1	229	—	1	3
13. 1.15....			35	—	226	—	—	26
14. 1.15....			46	—	229	1	1	2
15. 1.15....			47	—	212	4	4	20
16. 1.15....	9	273	57	—	216	—	—	7
				1		5	6	58
17. 1.15....			45	—	206	—	—	32
18. 1.15....			34	—	225	—	—	15
19. 1.15....			30	—	233	—	—	7
20. 1.15....			33	1	251	1	2	1
21. 1.15....	10	301	31	—	270	—	—	11
				1		1	2	66
22. 1.15....			30	—	274	—	—	7
23. 1.15....			30	—	259	—	—	24
24. 1.15....			30	—	238	—	—	26
25. 1.15....			37	—	222	—	—	18
26. 1.15....	11	264	33	—	231	—	—	23
				—		—	—	98
27. 1.15....			35	—	250	—	—	16
28. 1.15....			42	—	252	—	—	12
29. 1.15....			41	—	257	1	1	16
30. 1.15....			42	—	250	—	—	17
31. 1.15....	12	271	33	—	238	—	—	25
				—		1	1	86
1. 2.15....			39	—	212	—	—	26
2. 2.15....			34	—	236	—	—	7
3. 2.15....			34	1	221	—	1	37
4. 2.15....			26	1	241	—	1	8
5. 2.15....	13	251	20	—	231	—	—	16
				2		—	2	94
6. 2.15....			17	2	211	2	4	21
7. 2.15....			16	—	191	—	—	21
8. 2.15....			18	—	178	—	—	19
9. 2.15....			25	—	208	—	—	—
10. 2.15....	14	222	22	2	200	—	2	15
				4		2	6	76
11. 2.15....			23	—	201	—	—	11
12. 2.15....			14	1	193	—	1	17
13. 2.15....			18	—	184	—	—	22
14. 2.15....			14	—	177	—	—	20
15. 2.15....			12	—	181	—	—	5
				1		—	1	75

Table E (III).

LABORATORY DIP.

TICK KILLING OBSERVATIONS.

Every 5 days. 0.128 per cent. Arsenical Solution, with addition of Soft Soap and Paraffin.

DATE.	EFFECT OF DIPPING.	BRUSH.				UNDER TAIL.				
		No.	Number Initially Present.	Number which Attached.	Number Killed.	Percentage Killed.	Number Initially Present.	Number which Attached.	Number Killed.	Percentage Killed.
7.12.14 to 12.12.14	1	38	38	4	5.2	57	50	51	47.6	
13.12.14 to 17.12.14	2	59	3	21	33.8	50	58	58	53.7	
18.12.14 to 22.12.14	3	30	84	5	4.3	50	82	44	33.3	
23.12.14 to 27.12.14	4	92	16	32	29.6	88	59	85	57.8	
28.12.14 to 1.1.15	5	38	44	15	18.2	62	117	64	35.7	
2.1.15 to 6.1.15	6	67	29	15	15.6	115	188	108	35.6	
7.1.15 to 11.1.15	7	63	16	23	29.1	195	125	188	58.7	
12.1.15 to 16.1.15	8	47	34	24	29.6	127	73	128	64.0	
17.1.15 to 21.1.15	9	56	9	11	16.9	72	153	81	36.0	
22.1.15 to 26.1.15	10	40	56	17	17.7	144	141	157	55.0	
27.1.15 to 31.1.15	11	79	38	25	21.3	128	85	140	65.7	
1.2.15 to 5.2.15	12	88	14	12	11.7	73	80	82	53.6	
6.2.15 to 10.2.15	13	67	57	34	27.4	71	117	92	48.9	
11.2.15 to 15.2.15	14	87	6	15	16.1	96	59	96	61.9	
		Average percentage		48.2	Average percentage		1444	Average percentage		50.5

Table E (II').
CONTROLS.
Dipped in Water only.

Every 5 days.

DATE.	EFFECT OF DIPPING.	BRUSH.				UNDER TAIL.				UNDER TAIL AND IN BRUSH.		
		No.	Number Initially Present.	Number which Attached.	Number Dead.	Percent- age Dead.	Number Initially Present.	Number which Attached.	Number Dead.	Percent- age Dead.	Missing and Engorged.	Percent- age of Missing and Engorged.
7.12.14 to 12.12.14	1	26	32	1	1	1.7	79	78	—	—	57	26.5
13.12.14 to 17.12.14	2	40	16	6	6	10.7	134	4	—	—	62	31.4
18.12.14 to 22.12.14	3	32	45	1	1	1.3	127	47	5	2.2	27	10.7
23.12.14 to 27.12.14	4	62	20	—	—	—	166	56	2	0.9	135	44.4
28.12.14 to 1.1.15	5	35	25	3	3	5.0	156	37	4	2.0	73	28.8
2.1.15 to 6.1.15	6	54	15	1	1	1.4	170	54	1	0.4	53	18.0
7.1.15 to 11.1.15	7	55	1	1	1	1.7	212	57	—	—	105	32.3
12.1.15 to 16.1.15	8	36	31	1	1	1.4	217	30	5	2.1	58	19.0
17.1.15 to 21.1.15	9	57	4	1	1	1.6	216	65	1	0.3	66	13.4
22.1.15 to 26.1.15	10	31	7	—	—	—	270	13	—	—	98	30.5
27.1.15 to 31.1.15	11	33	10	—	—	—	231	27	1	0.3	86	28.5
1.2.15 to 5.2.15	12	33	7	2	2	5.0	238	44	—	—	94	29.1
6.2.15 to 10.2.15	13	20	9	4	4	13.7	231	30	2	0.7	76	26.2
11.2.15 to 15.2.15	14	22	5	1	1	3.7	200	5	—	—	75	32.3
		Average percent- age		Average percent- age		3.3	Average percent- age		Average percent- age		Average percent- age	
		253		616				0.6				26.5

Total number of Ticks under Observation... 869.

Table E (V).

SUMMARY.

0.128 per cent. Arsenite.

DIPPED LOT.

Date.	BRUSH.		UNDER TAIL.	
	Percentage of Ticks Killed.	Number of Ticks under Observation.	Percentage of Ticks Killed.	Number of Ticks under Observation.
2.12.14 to 15.2.15	10.3	482	50.5	1444

CONTROLS.

Date.	BRUSH.		UNDER TAIL.		BRUSH AND UNDER TAIL.	
	Percent-age Dead.	Number under Observation.	Percent-age Dead.	Number under Observation.	Percent-age Missing and Engorged.	Number of Ticks under Observation.
7.12.14 to 15.2.15	3.3	253	0.6	616	26.5	869

Isolation and Description of a Bacterium causing oxidation of Arsenite to Arsenate in Cattle Dipping Baths.

BY

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Biochemist, Division of Veterinary Research.

From the Onderstepoort Laboratories for Veterinary Research.

Pretoria, August, 1917.

Isolation and Description of a Bacterium causing oxidation of Arsenite to Arsenate in Cattle Dipping Baths.

By HENRY H. GREEN, D.Sc., Biochemist, Division of Veterinary Research, Onderstepoort. (Received August, 1917.*)

THE fact that arsenical dipping baths are prone to undergo oxidation, whereby the active tick-destroying sodium arsenite is converted into the less toxic arsenate, has been recognized for several years. Brännich¹ first called attention to this fact in 1909, and Fuller² in 1911 independently established the same phenomenon, further showing that it was to be attributed to bacterial agency. Since then Williams³ in 1913; Lewis,⁴ Brännich and Smith,⁵ Holborow,⁶ in 1914; and Chapin⁷ in 1915, have contributed studies of the oxidative changes occurring in tank fluids. Chapin's paper gives a considerable amount of detailed information concerning the behaviour of tanks in the field.

Although the accumulated data leave no room to doubt that the oxidation process is affected by bacteria, no isolation or description of the causal organism has yet been recorded, and it is therefore considered of interest to place on record a brief description of a vigorously oxidizing organism which we have isolated from the dipping tank at Onderstepoort.

An organism which effects the reverse change and reduces arsenate to arsenite, and several other organisms which are capable of tolerating fairly high concentrations of arsenic, but which neither oxidize arsenite nor reduce arsenate, have also been isolated, but their description is being postponed until time is available for their study.

Isolation of the oxidizing organism presented no particular difficulty, yielding readily to a combination of enriching and plating methods. A preliminary plating of the tank fluid on nutrient agar showed a bacterial count of about two hundred million organisms per c.c., but the flora appeared to be so varied that little was to be gained by picking off colonies for individual testing. From a plate five days old, at a dilution of one in ten million, twelve colonies as varied as possible in appearance were removed and inoculated into sterilized tank dip. In no case, however, was oxidation reinduced, and the attempt to pick out from first plating on the "chance method" was therefore abandoned.

* Read before the South African Association for the Advancement of Science at Stellenbosch, July, 1917, and given in abstract in the *South African Journal of Science*.

¹ Australian Association Adv. Science, 1909.

² Bureau of Animal Industry Circular 182, 1911.

³ *Agricultural Journal*, Union of South Africa, January, 1913.

⁴ *Agricultural Journal*, Union of South Africa, May, 1914.

⁵ *Queen-land Agricultural Journal*, 1914, Vol. 2, Part 1.

⁶ *Rhodesia Agricultural Journal*, 1914, Vol. 2.

⁷ Bulletin of U. S. Dept. Agriculture, No. 259, July, 1915.

A suitable enriching medium was then sought—one which would allow of rapid oxidation, permit the oxidizing organisms to compete successfully with other bacteria present, and neither interfere with the simple titration of arsenic by iodine nor be susceptible of rapid change of reaction. It was soon found that a dilute decoction of stable manure containing arsenite, and suitably bufferized with potassium phosphate, served the purpose admirably. Preliminary experiments, which need not be detailed here, together with a critical survey of the earlier literature on oxidation under field conditions, suggested that the oxidizing organism, though very active, was one which multiplied relatively slowly. It was also observed that any large quantities of easily decomposed organic compounds favoured development of organisms which were capable of tolerating arsenite, but not of oxidizing it.

Attenuation of medium was therefore considered as a probable method of limiting the field of operation of rapidly growing competitors without penalizing the oxidizing organism itself.

Stock solutions of mineral mixture and dung decoction were made up as follows:—

A.—Mineral Mixture:

Sodium arsenite equivalent to As_2O_3	0.2 per cent.
Sodium arsenate equivalent to As_2O_3	0.2 „
K_2HPO_4	0.2 „
MgSO_4 and CaCl_2	0.01 „
FeCl_3	trace.

The whole neutralized to phenol phthalein.

B. Dung Decoction:

20 grm. of dried ground stable manure, boiled for twenty minutes with one litre of water, filtered, and sterilized. Such infusion usually contained 0.1 to 0.2 per cent of dissolved organic matter.

On mixing A and B in varying proportions it was soon found that 9: 1, after inoculation with tank dip, was almost as rapidly oxidized as mixtures containing higher amounts of dung decoction. This dilution, corresponding to mineral mixture containing .01 to .02 per cent. of organic matter, and a moderate amount of arsenite, was considered as likely to permit, under favourable conditions of aeration, the enriching of the oxidizing organisms at the expense of undesirable aliens. As a matter of fact, in the light of subsequently ascertained knowledge of the habits of the organism, better enriching methods might be suggested. Mineral mixture containing .005 to .01 per cent. of peptone or dung, .05 per cent. of nitrate, and 0.5 per cent. of arsenite, would probably be quicker and enable all organisms of low tolerance to be eliminated at a single sub-inoculation. If the organisms are already numerous in the tank direct plating on to arsenite agar *may, perhaps*, be sufficient, but in general such plates are overcrowded with non-oxidizing species and discrimination is not easy. In the isolation process here adopted the mineral mixture dung decoction was distributed in 200 c.c. quantities in bolt-head culture flasks of 14 c.m. diameter, plugged, and sterilized. After inoculation with 2 c.c. of dip (1 per cent. inoculum), 10 c.c. quantities were withdrawn from day to day and titrated with iodine in presence of bicarbonate in

the usual way. Oxidation was found to be completed in about ten days. From the fully oxidized medium fresh sub-inoculation into sterile media was practised. After each successive sub-inoculation oxidation proceeded slowly at first, then gathered speed, giving a titration curve suggestive of a logarithmic rate of growth of the oxidizing bacteria. The rapidity of oxidation increased with each sub-inoculation, indicating an enriching of the medium in the desired organisms, and after the fourth transfer the rate of oxidation at the end of the fourth day, at 27° C., corresponded to about 1 mg. per c.c. per hour. This was taken as evidence that the organisms were present in sufficient numbers for plating out, and from this flask, therefore, plates of nutrient agar, nutrient agar containing 0.2 per cent. sodium arsenite, and dung decoction arsenite medium set with 2 per cent. of agar, were poured in varying dilutions. In the course of a few days various colonies appeared on all the plates, and after a week were so numerous that only dilutions corresponding to one per million, or less, could be used for picking off isolated colonies. Superficial examination indicated that several types of organism were still present, and the various colonies were therefore tested by inoculation into sterilized dung decoction arsenite medium—10 c.c. contained in 50 c.c. Erlenmeyer flasks. From an arsenite agar plate sixteen colonies, judged from their macroscopical and microscopical appearance to include every type present, were picked off. Of these, five were found to oxidize arsenite with great rapidity, while the remaining eleven effected no oxidation at all, even after standing for some weeks. The eleven non-oxidizing colonies comprised at least two morphologically distinct types, a fairly large coccus, and a motile coli-like bacterium. Examined in hanging drop, two out of the five oxidized test media appeared to contain only one type of organism—non-motile rods, showing a beaded structure on staining with methylene blue. The other three showed the presence of this same organism but were impure. On sub-plating a yellow coccus and a motile bacterium were separated, but, since neither of these displayed any oxidizing activity, they were laid aside for the time being. Taking these tests, together with the negative character of single colony testing from plates poured direct from the dip, it appeared that, in this dip at least, only one type of oxidizing organism had to be dealt with, and not several as had at first seemed conjecturally probable. Subsequent platings from the same tank on arsenite agar gave the same result. Only one organism was definitely detected. This grew moderately well in nutrient broth and on nutrient agar slopes, with or without incorporation of sodium arsenite in the medium. After two successive sub-platings on arsenite nutrient agar to ensure the purity of the culture the organism was kept going alternately on nutrient agar slopes and in dung decoction arsenite.

CHARACTERISTICS OF THE ORGANISM.

Morphology: The organism is a bacterium of polymorphic character—bacterium in the sense that it is non-sporulating. Although vigorous gyratory or molecular movement is shown, true relative motion could not be discovered at any stage of growth, and the strain here described is therefore put down as non-motile. It would appear,

however, that motility is a variable characteristic, since on one occasion practically all the individuals in a dung decoction young culture from a single colony were motile. An agar slope taken from this culture showed motility during the first forty-eight hours of growth, but on sub-culturing on to a second slope the motility disappeared by the time visible growth occurred and could not be restored. The precise conditions determining the production of motile forms have, however, not been specifically investigated, and our observations have been confined to a comparatively small number of colonies from only a few separate platings. During the time motile forms were under observation several attempts were made to determine the number of flagella. These, however, were difficult to stain, and on the two occasions upon which staining was successful detached flagella appeared in the neighbourhood of the bacterial bodies, making it impossible to ascertain the proper number attached. The organism is suggested as possessing several long easily detached flagella, probably peritrichic. The strain we are describing here may have been motile originally, and have lost its motility in the course of continued laboratory cultivation.

The typical form as occurring in young broth cultures and on agar slopes is rod-shaped—1 to 3 μ in length and 0.3 to 0.6 μ in breadth, i.e. with dimensions similar to *B. coli*, but with the majority of forms more slender. Oval forms can also occur, and they almost invariably show bipolar staining. The rods generally show bipolar staining, or a beaded appearance, which is very characteristic and reminiscent of *corynebacterium diphtheriae* or *B. pestis*. The granules or metachromatic bodies are very well marked and need no special staining methods for their detection. Aqueous methylene blue, Bismarck brown, dilute carbol fuchsin, carbol thionin, all bring out the structure well. In living unstained hanging drop preparations the beaded appearance can be detected as alternate light and dark bands. Sometimes the arrangement of granules suggests endospores, but the low resistance to heat and the fact that the structure is independent of age negatives this. Within our experience the beading has been found so constant a feature that it serves as a useful diagnostic.

In older cultures involution forms may be seen. The secondary growth of scum colonies and stalactitic threads which frequently form in bouillon cultures a few weeks old commonly show a variety of forms—rods lengthened up to 6 μ and thickened to 0.8 μ , frequently forked and budded, and sometimes enlarged at the ends; rods sometimes swollen into pear-shaped masses, sometimes pointed at one end like a cigar. The involution forms also show a beaded structure, staining alternately as light and dark bands. Some rods scarcely stain at all or only show fragmentary granular bodies. Clumps of roundish deeply staining bodies also occur.

Staining: Good with all ordinary stains. Not acid-fast. Gram-negative.

Oxygen Requirements.—The organism must be described as aerobic. In presence of air growth is good in suitable media. In dung decoction arsenite the rate of oxidation depends upon the surface exposed to the air.

In bouillon under strict exclusion of air (Wright-Burri method) growth is negligible—at most exceedingly slight and not enough to justify the designation "facultative." In the presence of nitrate,

growth, accompanied by denitrification, occurs under anaerobic conditions. Arsenate, however, does not appear to be able to take the place of nitrate, and, although oxidation of arsenite to arsenate is effected under aerobic conditions, the reverse change (reduction) is not effected under anaerobic conditions.

Temperature: Growth in bouillon appears to be best at about 27° C., but is not much inferior at 22° and at 37° C. In general, adverse influences such as unfavourable reaction and high concentration of arsenite operate more powerfully at 37° C. than at 27° C., and an inoculation into 0.2 per cent. arsenite bouillon may sometimes fail at 37° C., though taking well at 27° C. At 15° C. growth is notably slower. The thermal death-point is about 55° C. to 58° C. Ten minutes' heating at 60° C. is sufficient to sterilize a freshly inoculated bouillon culture, whereas heating from 50° to 55° C. has little or no effect on subsequent growth. In presence of arsenite the thermal death-point may be lower.

Reaction: In bouillon growth is best at about + 10, the reaction most generally favourable to bacterial development. At -2 and + 30 it is stopped. Bouillon, however, owing to its high and often variable buffer effect, is not a good medium in which to determine reaction limits, and the use of Fuller's scale of course give very little information about the true reaction. The effect of reaction proper, i.e. hydrogen ion concentration, will be discussed more fully in dealing with the range over which oxidation takes place.

MEDIA.

Bouillon: In nutrient broth growth is good, but slow when compared with a rapidly growing organism like coli. In twenty-four hours growth is scarcely visible, but in three days at 27° C. turbidity is fairly marked and a slight sediment forms. After about a week the sediment is heavy, coherent, somewhat viscid, and ropy, while the bouillon itself is practically clear. As time goes on the sediment becomes compact and cannot be distributed by shaking, while a surface pellicle frequently forms, upon which round colonies may grow and from which stalactitic threads may grow downwards. The presence of a little nitrate in the bouillon usually increases considerably the rate of growth. There is little or no change of reaction during growth. Even after the lapse of several weeks the titration figure for the clear bouillon, pipetted off from the sediment, is practically the same as the initial figure.

Ammonification in Bouillon: In the generally accepted sense this does not occur; 100 c.c. of bouillon, six weeks after inoculation, only showed 4 mg. of ammonia above the uninoculated control.

Peptone: In peptone water growth is similar to that in bouillon.

Indol Production: Negative.

Sulphuretted Hydrogen Production: Negative.

Odour: Practically none, and in any case uncharacteristic.

Hay Decoction: In 1 per cent. hay infusion growth is fairly good, and in general of a more filamentous character than in bouillon.

Synthetic Media: Poor or altogether absent. No appreciable growth in Uschinsky solution or in Cohn's solution.

Sugar Bouillon: In bouillon containing either glucose, lactose, or saccharose, growth is much the same as in ordinary bouillon. There is no fermentation and no change of reaction. Acid production was checked by titration after three, seven, and fourteen days' growth, but the final reaction did not vary more than ± 3 from the original $+ 10$. In fermentation tubes turbidity occurred only on the open side, indicating the dominantly aerobic character of the organism, and although a heavy sediment collected at the bend there was no gas production.

Glycerine Bouillon: No acid production.

Starch Bouillon: 0.05 per cent. starch remained unchanged for weeks.

Arsenite Bouillon: Growth in 0.2 per cent. arsenite bouillon is almost as rapid as in ordinary bouillon. With increasing concentrations of arsenite growth is gradually retarded. The limits of tolerance for arsenite, and the depression of activity were worked out in dilute dung decoction and are given below. Oxidation of arsenite is less rapid and less complete in bouillon than in dung decoction mineral mixture, although growth is more abundant, and bouillon is not a suitable medium in which to study oxidation.

Nitrate Bouillon: Growth is more vigorous than in ordinary bouillon and denitrification takes place with evolution of gas. In 0.3 per cent. nitrate bouillon distinct turbidity usually appears in twenty-four hours, when inoculation is effected with 1 c.c. of an oxidizing dung decoction medium containing a few million organisms per c.c. On the third day turbidity is very marked and frothing is noticeable. Before this a faint nitrite reaction may or may not be given. After about six days frothing subsides and a heavy sediment settles, leaving the upper regions of the test-tube comparatively clear. Denitrification is then complete, neither nitrite nor nitrate reaction being given, and the reaction is alkaline to phenol-phthalein. With higher concentrations of nitrate denitrification is not always completed, but as a rule anything up to 0.5 per cent. disappears in about a week.

This denitrification power is interesting, since *a priori* one might have expected that an organism capable of reducing nitrate would reduce arsenate to arsenite rather than oxidize arsenite to arsenate. The anomaly, however, is more apparent than real, and it may be recalled that Jensen found that denitrifying bacteria are usually particularly rich in peroxidase.

The organism, when kept going on agar slopes sub-inoculated at frequent intervals in the laboratory, may fall off in denitrifying power, but can usually be rejuvenated by passage through the dung decoction arsenite medium. Tests for denitrification are best carried out by inoculation from an actively oxidizing culture.

Litmus Milk: Unchanged. No proteolysis and no change of reaction within a month. At the end of this time some of the tubes showed a bleaching of the litmus in the lower regions of the liquid, suggesting a slow process of reduction.

Solid Media: On nutrient agar plates, poured at dilutions to show about 50 final colonies, and incubated at 27° C., growth is visible to the naked eye, or can be discerned with an hand-lens, about the third or fourth day. On the sixth day the surface colonies may be about

0.5 to 1 mm. in diameter, and show up as uncharacteristic dirty white or greyish dull-glistening pinheads. Sub-surface colonies are smaller, yellowish, or brownish, and generally lenticular with sharply pointed ends. A variety of shapes may, however, be shown, triangular stars being quite common. Microscopically the colonies are entire, granular, faintly yellowish, becoming darker, especially in the centre, as they grow older and denser. On the whole the colonies resemble somewhat those of the colon group, but grow very much more slowly. As time goes on the sub-surface colonies remain small, while the surface colonies may increase up to about 2 mm. in diameter, remain round and compact, and darken in colour.

Arsenite Agar: Colonies on ordinary nutrient agar to which 0.2 per cent. sodium arsenite has been added are similar to those on ordinary agar, but they grow even more slowly. After about ten days, however, there is little to choose between arsenified and unarsenified plates. In this respect they differ very much from ordinary strains of coli, which, so far as we have observed, are very much restricted by the presence of arsenite. The strain of coli used for comparison failed altogether on 0.2 per cent. arsenite agar, and was markedly inhibited on 0.05 per cent.

Agar Shake Cultures: Growth takes place on the surface and upper few millimetres only. Below this growth is scarcely appreciable. Sugar agar shake cultures are much the same and no gas production occurs. In 0.3 per cent. nitrate agar shake cultures gas production is evidenced by the bursting and cracking of the agar in the course of a few days. The cracking commences from the top downwards, suggesting the favourable influence of air upon growth even in the presence of nitrate.

Agar Stab Cultures: On the surface, dense, slightly raised, more or less circular growth occurs, white or greyish white, reaching a diameter of about 8 mm. in two or three weeks. Growth along the track of the needle is fairly good to a depth of about 1 c.m., and is tuberculate in character. Below this, growth is very faint and filiform.

Agar Slopes: Inoculated from a bouillon culture and incubated at 27° C., the growth is relatively slow but good, appearing as a fairly dense, smooth, slightly raised streak, at first keeping closely to the track of the stroke.

The final appearance is like that of *B. coli*, except that, owing to its slower growth, the mass is more compact and not so soft. After a few weeks the growth darkens in colour and takes on a faint brownish tinge. If one slope be heavily inoculated from another a good colic-like growth is obtained in a few days. If the stroke be drawn from a dilute medium (oxidation test flask) where the organisms are fewer, the growth at first appears as a multitude of small colonies which finally fuse to give an even growth of comparatively dry character.

The water of condensation becomes turbid and soon deposits a slimy sediment.

On 0.2 per cent. arsenite agar slopes the growth is considerably slower, and a week may elapse before it is at all heavy.

Gelatine: On nutrient gelatine growth is much slower, and as a rule only surface colonies appear on plates. More frequently gelatine plates fail altogether, and for this reason gelatine is not suitable for

isolation purposes. If a plate after setting is stroked with a lightly infected needle much better surface colonies are obtained. These, though similar in appearance to those on agar, do not reach so great a size, but remain small, compact, convex, and almost perfectly white.

On gelatine slopes stroked in the ordinary way from bouillon growth is very slow, and nothing at all may appear for a week or more. Growth then frequently begins as a multitude of small colonies, which finally fuse together to give a flat opaque streak, dense, white, and glistening, but thin and poor in comparison with an agar slope. Gelatine stab cultures were also poor, growth only occurring as a small opaque disc on the surface with hardly any development along the track of the needle.

No liquefaction occurs even after six weeks.

Potato: Growth evidently depends considerably on the reaction and may sometimes fail, particularly if the inoculation be light. Good growth, however, can be obtained, usually most readily on alkalized potato. For the first five or six days it may be too slight to detect or approximate too closely to the colour of the potato. Thereafter, however, a soft glistening growth appears, growing thicker and darker in the course of about ten days. In three weeks the streak is heavy, soft, and wet, with a slight yellowish brown tinge, but not in any way characteristic.

Pathogenicity: Apparently non-pathogenic for rabbits and guinea-pigs when given by intraperitoneal or subcutaneous injection.

SPECIAL CHARACTERISTICS.

Tolerance for Arsenite: The organism can oxidize within wide limits of concentration of arsenite, although in general the lower the concentration the more rapid is the oxidation. It can remain alive for a considerable length of time in media containing arsenite equivalent to 1 per cent. As_2O_3 , and is capable of slow growth and of effecting slow oxidation in concentrations as high as 0.8 per cent.—a concentration which completely inhibits the majority of the commoner bacteria which we have had an opportunity of testing. In passing, it is interesting to note that bacteria behave very differently in regard to their tolerance for arsenic. *B. subtilis*, for instance, is completely inhibited in .05 to 0.1 per cent. arsenite bouillon. *B. coli* grows freely in 0.02 per cent., is notably restricted by 0.05 per cent., and practically suppressed by 0.1 per cent. *B. pyocyaneus* may at first be restricted by 0.2 per cent., but readily accustoms itself to this and can be educated up to at least 0.5 per cent. We have now several different organisms isolated from a dipping tank, not yet fully investigated, which can grow freely in media containing arsenite equivalent to 0.5 per cent. As_2O_3 . One of these, which reduces arsenate to arsenite, can tolerate up to 1 per cent. Another, which appears to be a variety of *B. fluorescens non-liquefaciens* can tolerate up to 1 per cent., but is capable neither of oxidizing arsenite nor reducing arsenate. It is possible that tolerance to arsenite might be turned into a useful diagnostic in general bacteriology, although, from a few observations we have made, it seems possible that a great number of different organisms could be “educated” up to a tolerance for concentrations considerably above those which they can normally stand.

Thus the strain of *B. coli* worked with, which on first trial was markedly inhibited by 0.05 per cent. As_2O_3 , can now, after successive sub-inoculations into gradually increasing strengths of arsenite bouillon, grow as well in 0.1 per cent. as it formerly could in 0.05 per cent., and is even capable of very slow growth in 0.2 per cent. Bacteria in general tolerate arsenate very much better than arsenite, and *B. coli* can easily stand 0.5 per cent. As_2O_3 in the form of the more highly oxidized compound. We have not yet had an opportunity of comparing the strain of *B. fluorescens* isolated from the dipping tank with a typical ordinary strain, but it seems probable that this organism would be a useful one upon which to work out the question of "acquired tolerance."

The following table indicates the behaviour of the oxidizing organism in different concentrations of arsenite. 200 c.c. quantities of dilute dung decoction mineral mixture were fortified with sodium arsenite to concentrations 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 per cent. As_2O_3 respectively, neutralized to phenol-phthalein and sterilized. Each was then inoculated with 2 c.c. of an actively oxidizing pure culture from a dung decoction medium, and at varying intervals 10 c.c. quantities were withdrawn for analysis.

Flask No.	Concentration of Arsenite ; per cent. As_2O_3 .							
	Initial.	2 days.	4 days.	6 days.	8 days.	10 days.	22 days.	24 days.
1	0.10	0.09	0.05	0.01	Nil.	—	—	—
2	0.20	0.19	0.16	0.14	0.126	0.107	0.102	0.102
3	0.40	0.40	0.397	0.338	0.303	0.285	0.270	0.270
4	0.60	0.60	0.60	0.60	0.594	0.590	0.490	0.486
5	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
6	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

In addition to titrating for arsenite, tests of reaction were made and it was found that, as oxidation proceeded, it was accompanied by increasing acidity to phenol-phthalein—presumably by conversion of arsenite which is alkaline to this indicator into arsenate which, like phosphate, may be acid. To methyl orange the reaction was of course always alkaline. On testing with neutral red it was found that for Flask No. 2 the turning point occurred about the sixth or eighth day. After this the reaction was indicated as distinctly acid to neutral red, and it is interesting to note that this is just where oxidation begins to slow down. Between the second and the sixth day, while the reaction was still alkaline to neutral red, the concentration falls from .19 to .14, i.e. falls .05 per cent. Between the tenth day and the twenty-second day, during which time the reaction was faintly acid to neutral red, the fall is only from .107 to .102, i.e. .005 per cent., or only one-tenth as much. In the two days following no oxidation at all is recorded. On bringing the reaction back to phenol-phthalein neutrality, however, oxidation was vigorously resumed, and the arsenite concentration fell from .102 to zero in two days. The behaviour in Flask No. 3 was similar. Oxidation, which had apparently stopped at some time between the tenth and twenty-second day, was promptly resumed after

adding sufficient dilute caustic soda to bring the reaction back to neutrality to phenol-phthalein, the arsenite concentration then falling from 0.270 to 0.061 per cent. in six days. Complete oxidation was also effected in Flask No. 4 by periodically adding sufficient caustic soda to bring the reaction distinctly alkaline to neutral red, the process taking place in stages of increasing rapidity as the concentration of the arsenite fell. Flasks Nos. 5 and 6, which, in spite of favourable reaction had failed to oxidize on the first inoculation were then re-inoculated from Flask No. 4. Flask No. 6 again failed to respond, but in Flask No. 5 oxidation was induced, proceeding very slowly at first but gathering speed each time the change in hydrogen ion concentration was rectified by addition of alkali, and being finally completed in about two months. There is here the suggestion that the organisms of the original inoculation from 0.2 per cent. arsenite, and probably habituated to that concentration by several generations of growth, had raised their tolerance by growth in 0.6 arsenite up to the point of commencing operations in a concentration as high as 0.8 per cent. As_2O_3 . Arsenite equivalent to 1 per cent. As_2O_3 , however, appears to represent the upper limit of concentration tolerated by this strain. Whether it would be possible to raise the tolerance still higher is doubtful. We have inoculated several samples of fresh dip from different tanks into media of varying arsenical concentration, but although it was always found quite easy to secure oxidation from 0.6 per cent. As_2O_3 downwards we have not succeeded in obtaining it at any concentration above 0.9 per cent.

In regard to the influence of concentration of arsenite upon the rate of oxidation it is quite certain that, as the concentration rises, the absolute rate falls. This is apparent from the data given above. The difference between the rates of oxidation in initial concentrations of 0.2, 0.4, and 0.6 per cent. As_2O_3 is quite marked. After four days the reaction in Flasks Nos. 2, 3, and 4 was still favourable for oxidation, yet in No. 2 the fall is from 0.2 per cent. to 0.16 per cent., or a total of 80 mg. As_2O_3 oxidized, while in No. 3 it is only from 0.4 to 0.397, or a total of only 6 mg. oxidized. In No. 4 oxidation is not yet noticeable.

Flasks Nos. 1 and 2 cannot be safely compared, since—although the rate of oxidation is suggested as greater in the first—the buffer effect of the medium was not high enough to absorb the acidity involved in arsenate formation, and so to maintain a hydrogen ion concentration favourable for continued growth and oxidation. By the sixth day both flasks had probably passed the turning point of neutral red, and slight error in initial neutralization may have vitiated the data. Subsequent tests, indeed, in which parallel flasks were maintained distinctly alkaline to neutral red throughout the whole period of oxidation, indicated that an inhibiting influence, due to the difference in concentration between 0.1 per cent. and 0.2 per cent. As_2O_3 was, though detectable, very slight.

An accurate curve showing the influence of concentration upon rate of oxidation has not yet been plotted. This would be troublesome to procure owing to the necessity for eliminating the factor of constantly changing reaction. Such a curve, even if plotted at approximately constant reaction, would probably vary with the hydrogen ion concentration adopted. It was, however, considered of interest to attempt to determine approximately the limits of reaction between which oxidation could take place.

HYDROGEN ION LIMITS OF OXIDATION.

A hydrogen electrode was not available for this work, and recourse had therefore to be taken to such indicators as were procurable. Methyl orange, methyl red, paranitrophenol, neutral red, and phenolphthalein were available and thymol-phthalein was easily prepared. Unfortunately α -naphthol-orange and α -naphthol-phthalein were not procurable, so that the range around P_H .8 had to be obtained by calculation. The medium used consisted of dilute hay decoction (0.2 per cent.), the buffer effect of which was found to be very low, and the pale colour of which did not interfere seriously with indicator tints, containing M/100 K_2HPO_4 , whose buffer effect is known, and sodium arsenite equivalent to 0.1 per cent As_2O_3 , which exercises a buffer effect constantly changing throughout the course of oxidation. 200 c.c. quantities of this were filled into bolt-head culture flasks of 14 c.m. diameter, neutralized to phenolphthalein, plugged and sterilized. The titration range of a sample flask was then determined with respect to the indicators available, and from the known hydrogen ion concentration of the turning points—as given in the table of Salm and Sørensen compiled by Bayliss—and careful matching of indicator tints in decimolar phosphate mixtures of known H^+ ion concentration, the quantities of decinormal sulphuric acid and caustic soda required to bring to desired limits were approximately calculated. Between the limits registered by any two indicators it was assumed that the change in hydrogen ion concentration effected by addition of known quantities of acid and alkali to the medium was similar to the change effected in equivalent strengths of K_2HPO_4 . This is not quite accurate, since the buffer effect of arsenite, arsenate, and phosphate are different, but since the chief buffer effect of the medium is due to K_2HPO_4 the method sufficed to provide a general range near enough for a preliminary test. Sufficient sterile acid or alkali was then added by means of sterile pipettes to bring the media to the desired reaction, and inoculation then effected with 2 c.c. of an actively oxidizing pure culture from a similar medium. 10 c.c. quantities were withdrawn at intervals and titrated for disappearance of arsenite and change of reaction.

The following data, in which the H^+ ion concentrations given are only intended as rough approximations, illustrate the behaviour in one series of tests:—

Approx.	Initial.	After 3 Days.		After 5 Days.		After 7 Days.		After 9 Days.	
P_H .	As_2O_3 .	P_H .	As_2O_3 .	P_H .	As_2O_3 .	P_H .	As_2O_3 .	P_H .	As_2O_3 .
6.5	.100	6.5	.100	6.5	.100	6.5	.100	6.5	.100
7	.100	<7	.075	6.9	.061	6.8	.043	6.8	.041
8	.100	7.5	.053	7.1	.034	7	.020	6.9	.006
9	.100	8	.060	7.5	.022	7	Nil.	—	—
10	.100	9	.099	8	.092	7.5	.024	7.2	Nil.
11	.100	<11	.100	10	.100	<9	.095	8	.065

It would appear that P_H . 6.5 is definitely too acid and P_H . 11 definitely too alkaline for growth and oxidation—the oxidation

indicated as commencing about the seventh day in the last case was preceded by independent change of reaction, probably due to absorption of CO_2 from the air. P_H . 10 is suggested as about the limit of alkalinity at which oxidation or growth can occur.

The figures also suggest that the optimum reaction for growth and oxidation lies somewhere about P_H . 8, since the highest number is there recorded within the first three days. Oxidation is, however, also rapid at the neutrality point P_H . 7, although it is promptly checked by the change towards higher H^+ ion concentration associated with the oxidation process itself. By the seventh day oxidation has been brought to a standstill, while nearly half the arsenite remains unchanged—the buffer effect of the medium being too low to carry further conversion of alkaline arsenite into acid or neutral arsenate. At P_H . 9 the initial rate of oxidation is lower, but the subsequent oxidation much more rapid. By the third day the reaction has become more favourable and, owing to the initial higher alkalinity, this flask does not suffer from unfavourable approach to the acid turning point. Oxidation is therefore completed sooner than it is in the flask originally at P_H . 8, which, although starting off more favourably, ends up with a faint but unfavourable acid reaction. The flask starting at P_H . 10 shows very little oxidation by the third day, but by the fifth day the reaction has become more favourable and oxidation proceeds rapidly thereafter. Where a flask begins at a reaction on the alkaline side of phenol-phthalein the initial increase of H^+ ion concentration is probably hastened by the absorption of CO_2 from the air.

It is interesting to note that a very slight departure from true neutrality towards acidity is sufficient to bring oxidation to a standstill. We have estimated that a rise from neutrality P_H . 7 to faint acidity P_H . 6.8 is sufficient to inhibit the process. Oxidation will not take place in a medium adjusted to the turning point of paranitrophenol, and by knowing the whole titration range "methyl orange—neutral red—phenol-phthalein" of the medium it is easy to estimate the limit of acid inhibition as being above P_H . 6.6 and below P_H . 7. Further tests have shown that the limit of alkalinity lies between rose to phenol-phthalein and faint blue to thymol-phthalein, i.e. between P_H . 9 and about P_H . 10.5.

An inoculated flask of medium, similar to that used in the preceding tests, was maintained at very faint blue to thymol-phthalein by periodic addition of calculated quantities of $\text{N}/10$ alkali. Even after three weeks no oxidation whatever was registered. Kept at faint pink to phenol-phthalein oxidation was fairly rapid, though not so rapid as when kept just on the acid side of this indicator. When kept at rose-red to phenol-phthalein but colourless to thymol-phthalein oxidation proceeded very slowly in some cases, while inoculation failed in others. There can be little doubt, therefore, that the limit of alkalinity tolerated by the organism is represented approximately by P_H . 10. The *optimum* reaction for oxidation was more difficult to determine, since indicators of turning point intermediate between P_H . 7 and P_H . 9 were not available, but in media kept at -5 to neutral red but +2 to phenol-phthalein, and gauged to be about P_H . 8, oxidation was completed after light inoculation as before in six days, as against seven days in media maintained just on the alkaline side of neutral red or just on the acid side of phenol-phthalein.

Summarizing then, we may say that growth and oxidation proceed best in a faintly alkaline medium, but that differences are not strongly marked between the limits P_H . 7 to P_H . 9; that inhibition is marked the moment the true neutrality point (water) is passed on the acid side, and that activity is brought to a standstill at so faint a departure as P_H . 6.8; that inhibition is again marked as P_H . 10 is approached, and that an alkalinity higher than this arrests oxidation altogether.

Whether rate of growth and oxidizing activity run absolutely parallel in media of different reaction has not been specifically determined. Broadly speaking they do, and it is probable that the parallelism is fairly rigid, although sometimes a slight growth turbidity may be observed in media a shade too acid to permit of oxidation.

OXIDATION IN RELATION TO OXYGEN SUPPLY.

It has been already mentioned that oxidation is more or less proportional to the extent of surface of medium exposed to air. The following rough data illustrate the point:—

Medium: dung decoction 0.2 per cent., K_2HPO_4 0.2 per cent., arsenite equivalent to 0.1 per cent. As_2O_3 . 200 c.c. quantities, plugged, sterilized, and inoculated with 1 c.c. of actively oxidizing culture in medium of similar composition:—

- (a) Exposed in wide bolt-head culture flasks of 14 c.m. diameter. Oxidation complete in six days.
- (b) Exposed in tubes of 8 c.m. diameter. Oxidation equally rapid, and also complete in six days.
- (c) Exposed in tubes of 3 c.m. diameter. Oxidation slower, but complete in ten days.
- (d) Exposed in narrow-necked bottles of 1 c.m. diameter filled to the brim. Oxidation complete in twenty-five days.
- (e) Exposed in similar flasks filled to the brim, corked, and sealed to exclude air. Oxidation negligible after one month.

Beyond illustrating the increase in rapidity of oxidation with increase of air supply these figures have of course no significance, since the actual rate of oxidation depends partly upon the number of organisms present to carry out oxidation. With a limited supply of air oxidation is no faster with a heavy inoculation than with a light one. With a free supply of air the rate of oxidation is limited by light inoculation, as in cases (a) and (b). With a heavy inoculation greater use can be made of a liberal air supply. It may be mentioned that growth seems less dependent than oxidation upon air supply, and a fair multiplication appears to go on under conditions which allow of only very slow oxidation.

Oxidation in Presence of Nitrate.—If a medium be supplied with both arsenite and nitrate good growth can occur under anaerobic conditions, but the extent of oxidation is limited by the amount of nitrate present. With a liberal air supply, as in shallow layer, oxidation may be completed before denitrification is complete. With limited air supply oxidation is also limited and denitrification may be

completed before oxidation. Atmospheric oxygen is therefore necessary for oxidation in general, although it may be possible under some circumstances for the organism to effect slight oxidation of arsenite by reducing other compounds. As already mentioned arsenate can not take the place of nitrate, and can not serve as oxygen source under anaerobic conditions.

Vigour of Oxidation.—The oxidizing activity of the organisms in relation to their numbers is very high, and a large quantity of arsenite can be converted into arsenate in a medium very highly attenuated in respect to organic matter, provided the buffer effect is sufficiently high to prevent inhibition of oxidation by undue change of H⁺ ion concentration. In the dung decoction mineral mixture used for enriching the numbers of the oxidizing organisms relatively to irrelevant bacteria of the dip, the actual organic matter present was only .01 to .02 per cent. But still higher attenuations of organic matter are sufficient, and complete oxidation of 200 mg. of As₂O₃ can be secured within a fortnight in a medium containing only 1 mg. of organic matter. This may be readily demonstrated either with a pure culture or with the dip itself. In some of our tests 200 c.c. of the mineral mixture used in the original isolation experiments (omitting the dung decoction) was inoculated with 2 c.c. of fresh tank dip and oxidation found to be complete within twelve days. A further 200 c.c. (containing .2 per cent. As₂O₃) was then inoculated with 2 c.c. of this attenuated medium, and oxidation found to be complete within a month. The organic matter present in the tank dip, diluted down *ten thousand times* with mineral mixture, was thus sufficient to act as medium for the complete oxidation of 400 mg. As₂O₃.

A few tests were also carried out with a suspension of pure culture made by shaking up a fragment of growth from agar slope (taken on a platinum loop) in 10 c.c. sterile water. Using 1 c.c. of this opalescent bacterial suspension as inoculum into sterilized mineral mixture media containing known small quantities of organic matter the following results were obtained:—

Mineral Mixture and Peptone.	Sodium Arsenite as percentage As ₂ O ₃ .								
	Initial	4 days.	6 days.	9. days	12 days.	16 days.	20 days.	24 days.	36 days.
200 c.c. mineral mixture alone	·200	·200	·200	·198	·195	·162	·080	·027	Nil.
200 c.c. mineral mixture + ·0001 peptone	·200	·200	·200	·198	·193	·160	·076	·023	Nil.
200 c.c. mineral mixture + ·001 peptone	·200	·200	·198	·192	·180	·120	·048	Nil.	
200 c.c. mineral mixture + ·01 peptone	·200	·200	·198	·187	·105	Nil.			
200 c.c. mineral mixture + ·1 peptone	·200	·185	·106	Nil.					
200 c.c. mineral mixture sterile control	·200	—	—	—	·200	—	—	—	·200

From the data it is apparent that vigorous oxidation can take place in media containing very small amounts of organic matter. With .01 per cent. peptone oxidation is complete in sixteen days;

with .001 per cent. in less than twenty-four days. Below this oxidation still takes place and is complete in thirty-six days, even in the flask which received no addition of organic matter at all and was dependent upon the organisms actually introduced by the inoculum itself. It may be mentioned that the emulsion used was made from an old laboratory culture. With a young, freshly isolated culture it is probable that oxidation would have been more rapid. The inference from these data is not of course that the bacterium is autotrophic, but simply that its oxidizing activity is very high. In attenuated media the growth is very slight, and the real number of bacteria rises rapidly as the amount of organic matter increases, being vastly greater in 0.1 per cent. peptone than in 0.001 per cent.

The activity in attenuated media is, as will be shown later, in marked contrast to that of the reducing organism which requires a liberal supply of organic matter to effect reduction of arsenate to arsenite. In mixed culture (dip itself) a high proportion of organic matter favours reduction rather than oxidation.

Classification.—It will be cheerfully admitted on all sides that it is no easy matter to classify an organism when a bacterial collection is not available for direct comparison, and one is forced to rely upon text-book descriptions. Identification is usually simplified when definite pathogenic characteristics are demonstrable, but in the case of non-pathogenic organisms a text-book description of general cultural features and commoner biochemical characteristics may frequently cover two organisms, which in detailed comparative study prove to be different. In the present case the matter is further complicated by the fact that, apart from denitrification, the general biochemical features of the organism in question are somewhat negative in character and that the outstanding peculiarity is one in regard to which no investigations in general determinative bacteriology have been made. High tolerance for arsenite and capacity to oxidize this to arsenate are characteristics which have hitherto been ignored in bacterial classification. The result is that, even if the organism had already been described under guise of other activities, we could never be sure of identity without direct comparison of strains. The group number of our organism, according to the system of the Society of American Bacteriologists, would be 212.3331033. This at once suggests *Bacillus alcaligines* of Petruschky, but identity with this organism is excluded by direct comparison. The strain of *B. alcaligines* used for comparison could not effect oxidation, and could not even grow in bouillon containing 0.1 per cent. As_2O_3 as arsenite. Identity is further excluded by the absence of alkali production in media such as milk, by absence of H_2S formation, and by minor morphological and cultural differences. It may be, however, that the organisms are allied. Meanwhile we regard it as a new species, and, while recognizing that the tendency towards creating new names without strict exclusion of identity with earlier-described species, by elaborate comparison with classical strains, is a deplorable one and liable to introduce much confusion into determinative bacteriology, we consider that the novel characteristic of this organism in its behaviour towards arsenic justifies provisional christening. It may therefore be named *Bacterium arsenoxydans* until such time at least as the behaviour of other organisms of similar general characteristics receive closer study in relation to tolerance for and oxidation of arsenite.

SUMMARY.

An organism has been isolated from an arsenical dipping bath which oxidizes sodium arsenite to sodium arsenate with great vigour, even in mineral media containing only very small amounts of organic matter. It has also been detected in mixed cattle and horse faeces. It is the causal organism of deterioration in arsenical cattle dips, and so far as has been ascertained by limited observation of a few tanks it is the only one to which rapid deterioration is to be ascribed. Its dimensions are variable, usually 1 to 3 μ in length and 0.3 to 0.6 μ in breadth, slender rods predominating. Involution forms are larger and vary considerably in size and shape. It stains well with all ordinary stains, shows a beaded structure or bipolar staining, and is gram-negative. It is described as non-motile, but motile forms which readily lose their motility have been observed. It has been named *Bacterium arsenoxydans*, and would have the group number 212.3331033 in the classification system of the American Society of Bacteriologists. Apart from its denitrifying activity and its power to oxidize arsenite to arsenate, its characteristics are rather negative. Apart from the negative characters indicated by the group number it does not ammonify bouillon, nor produce alkalinity in milk media, produces neither indol nor sulphuretted hydrogen, nor any characteristic odour in bouillon. It grows either poorly or not at all in synthetic media such as Uschinsky's, Giltay's, or Cohn's. Growth on agar is slow, but in the course of a week is good; on gelatine poor and may fail; on alkalized potato slow and uncertain, but may be good; in organic media such as bouillon, peptone, hay infusion, is good but slow. Plate cultures on agar and agar slopes are not unlike those of *coli*, except that the growth is slower and more compact. It is differentiated from most of the commoner organisms by its high tolerance for arsenite and its capacity to oxidize this to arsenate. The limit of tolerance is about 1 per cent. As_2O_3 , and oxidation can proceed slowly in concentrations as high as 0.8 per cent. The rate of oxidation under suitable conditions of air supply and reaction increases as the concentration of arsenite decreases. At 0.2 per cent. As_2O_3 oxidation, after moderate inoculation into suitable media, may be complete in five or six days. Oxidation proceeds best in a faintly alkaline medium, being inhibited by very slight acidity, and rapidly coming to a standstill in neutral media unless the buffer effect is sufficient to absorb the change of H-ion concentration, accompanying the transformation of alkaline arsenite into neutral or acid arsenate. The reaction limits are approximately assessed as P_H . 6.8 to P_H . 10 or perhaps P_H . 6.6 to P_H . 10.5.

Although the organism does not grow under anaerobic conditions in bouillon, growth readily occurs in presence of nitrate. Arsenate can not take the place of nitrate, i.e. although arsenite is vigorously oxidized to arsenate under aerobic conditions the reverse change of reduction is not effected under anaerobic conditions.

Description of a Bacterium, Isolated from a Cattle Dipping Tank, which reduces Arsenate to Arsenite.

BY

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From the Onderstepoort Laboratories for Veterinary Research.

Pretoria, October, 1917.

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By HENRY H. GREEN, D.Sc., Biochemist, Division of
Veterinary Research, Onderstepoort.

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IN a previous paper¹ the causal organism responsible for oxidation of arsenite to arsenate in dipping tanks was described. The present communication deals with an organism capable of bringing about the reverse change, since isolated from the same source—the cattle dipping tank at Onderstepoort.

In the literature relating to the use of arsenical dipping tanks, reference is occasionally made (*l.c.* 1) to field observations of oxidation and reduction, and Lewis² noted that a dip which became oxidized when fully exposed to air in shallow layer might still be capable of reducing added arsenate when the air supply was restricted. No isolation or description of the organism, or organisms, responsible for reduction has yet been recorded, however, and the present communication therefore proposes to fill the gap.

Watkins-Pitchford³ in a private communication informed me that he had some years ago obtained evidence of arsenate reduction with *B. coli*, and mentioned that he thought many other organisms capable of reducing nitrate to nitrite might also reduce arsenate to arsenite; adding that his own early observations had never been completed for publication.

We have tried out the suggestion of *B. coli* on three different strains, two isolated by ourselves, and one obtained from the Medical Institute in Johannesburg, but in all three cases found that this organism was intolerant of arsenite, though fairly tolerant of arsenate; could only stand about 0.1 per cent. of arsenite, and could not reduce arsenate to any *significant* extent. In synthetic media the maximum reduction only proceeded to .005 per cent., as against .106 per cent. found by Pitchford. In glucose bouillon our strains of coli could reduce up to .05 per cent.—still relatively insignificant.

As will be shown later the reducing organism described here belongs to the colon-typhoid family, but its biochemical characters place it in the typhosus group, and with it reduction of arsenate can

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¹ Published in the present bound volume of the 5th and 6th Reports of the Director of Veterinary Research.

² Lewis: *Agricultural Journal*, Union of South Africa, May, 1914.

³ Director of the Medical Research Institute, Johannesburg.

proceed up to 1 per cent. As_2O_3 —ten times as high as with Pitchford's typical *coli*. There is, however, the suggestion that *slight* reduction may be effected by several members of the family, and even that vigorous reduction may not be confined to the particular organism dominant in the Onderstepoort tank and described here. It may be mentioned that although *coli* is regularly introduced into our Laboratory tank in the faeces of dipped animals, it appears to die out (or undergo metamorphosis?) in the presence of arsenite (0.2 per cent.) and does not remain a frequent inhabitant of the dip.

In regard to the relation between arsenate reduction and nitrate reduction we can definitely state that no necessary connection exists. We had started off with the supposition that such a relation was highly probable, but soon found that members of the putidum group, which are very abundant in dipping tanks and very tolerant of arsenite, can not reduce arsenate, although they very readily reduce nitrate. It happens that the arsenate reducing organism described in this paper also reduces nitrate, but the connection is not a general one.

The observations of Lewis suggested that reduction was brought about under more or less anaerobic conditions, but at the same time left the general conditions of life of the organism quite open. The exclusion of air operates against oxidation, and therefore leaves a clear field for the reverse reaction, but this, of course, does not mean that the organisms themselves thrive better under limitation of oxygen. As will be shown later, free aeration accelerates the growth of the reducing organism.

In attempting to isolate the reducing organism the dilute dung decoction arsenite-arsenate medium used in the enriching process for the isolation of the oxidizing organism was first tried, air being excluded in order to prevent the oxidizing organisms gaining the upper hand. Under these conditions, however, little or no reduction could be effected on inoculation with tank dip. The factor missing in this test, but occurring in most tanks under field conditions, was obviously the presence of small amounts of urine. On the addition of about 5 per cent. of urine to our dilute dung decoction mineral mixture, or on increasing the concentration of the dung decoction, and again inoculating with dip, reduction was found to proceed with moderate rapidity. The addition of larger quantities of urine rather militated against reduction, owing possibly to the high concentration of ammonia formed by decomposition and the predominance of organisms of an arsenic-resistant but non-reducing type. The presence of much urine in a medium is also objectionable from the point of view of arsenite determination by simple iodine titration, and a more suitable medium was therefore sought. It was soon found that reduction of added arsenate could be readily effected in a number of the commonly used synthetic media; that, for example, a mineral mixture containing ammonium salts as nitrogen source and glycerine or glucose or carbonaceous source, showed good bacterial growth and rapid reduction of added arsenate on inoculation with tank dip. An enriching medium was therefore constructed, of the following composition:—Glycerine 1 per cent., NH_4Cl .2 per cent., MgSO_4 .01 per cent., CaCl_2 .01 per cent., K_2HPO_4 .2 per cent., sodium arsenite equivalent to 0.1 per cent. As_2O_3 , and sodium arsenate equivalent to 0.3 per cent. As_2O_3 ; the whole brought just to the acid side of phenol-phthalein and sterilized. This medium is rather more acid to phenol-phthalein after

sterilization than before, but it remains faintly alkaline to neutral red. On inoculating with tank dip bacterial growth was rapid and maximum reduction was effected in a few days. A few tests were made under various conditions of aeration, and it was found that in this medium reduction took place in any depth of layer at all—as readily in a layer of 3 mm. depth as in glass tubes of 300 mm., and better than in large bottles filled right up to the neck. Further subinoculation was therefore carried out for the most part into test-tubes in the ordinary way. It was soon observed that although reduction was exceedingly rapid at first a definite limiting concentration of arsenite was soon reached. Whether the medium contained arsenate alone, or arsenate and arsenite in varying proportions, a concentration of about 0.2 per cent. As_2O_3 as arsenite was reached in about 48 hours. This rose to .26-.30 per cent. in the course of a week, after which no further reduction occurred. As will be shown later, however, .3 per cent. does not represent the upper limit of reduction, but merely the limitations of the medium. Determination of the rate of reduction provided a simple means of keeping track of the organism through successive changes of media. Successive subinoculation was practised at intervals of four days into tubes of the synthetic medium, rate of reduction being determined by iodine titration and type of flora by microscopic examination. After about half a dozen transfers the culture seemed to be dominated by one type of organism, an actively motile bacterium. Plating out was then resorted to, bouillon agar containing 0.1 per cent. of arsenite being used. Colonies appeared within a few days, and after the lapse of about a week the plates at dilutions below one per million were systematically examined. Only three different types of organism could be detected, an infrequent coccus form which on testing was found to possess no reducing power, an infrequent motile non-reducing organism which appeared to belong to the putidum type, and a motile bacterium which dominated the plate and appeared to be identical with the form dominating the medium from which they were poured. On inoculating single colonies of this back into the synthetic enriching medium already described, growth was found to be very feeble and very little development occurred unless the inoculation were a very heavy one. On supplementing the medium, however, with a small quantity of bouillon, dung decoction, or hay infusion, growth at once improved and the rapidity of arsenate reduction left no doubt that this organism was the one originally sought after. The curious behaviour in regard to the enriching medium, suggesting the necessity for some hormone which had possibly been carried over from the dip through the series of enriching subinoculations, or provided by the organisms growing commensally in the impure culture, is of some interest, but has not been further investigated. From the point at which this observation was made the synthetic reducing medium was modified for general use by the incorporation of 10 per cent. of a 2 per cent. filtered hay infusion or dung decoction. This small amount colours the medium slightly, but has no appreciable effect on the reaction end-point to indicators, or upon the iodine titration for arsenite. A small amount of peptone, or of bouillon (5 per cent.—10 per cent. of ordinary bouillon) is equally serviceable, and glucose is even preferable to glycerine. In 1 per cent. peptone water containing 2 per cent. of glucose, .2 per cent. K_2HPO_4 , .2 per cent. As_2O_3

as arsenite, and .2 per cent. As_2O_3 as arsenate, reduction may proceed up to .4 As_2O_3 per cent. in 24 hours.

The organism itself may now be described.

Morphology.—The organism is a bacterium in the sense that it is non-sporulating. It is vigorously motile, with long peritrichic flagella usually four to eight in number. In old cultures, especially in those containing high concentrations of arsenite, it may lose its motility. A non-motile culture usually recovers its motility on being put through the usual process of rejuvenation.

The bacteria are rod-shaped with rounded ends, but they may vary considerably in size, the dimensions of the typical form in young bouillon cultures being about 2μ in length by about 0.4μ in breadth. In older cultures the dimensions may range from 1μ – 6μ in length and 0.3 – 0.6μ in breadth, the rod-like type predominating with only occasional oval forms. In young cultures the rods are generally single, occasionally in pairs, but as the culture ages there is a marked disposition to chain formation, as many as ten or twenty rods being sometimes seen in one long chain. As these chains may be motile, they usually appear in hanging drop as rapidly-swimming enormously lengthened bacteria, but a stained preparation at once shows them up for what they are. Older non-motile forms may occasionally show a slightly beaded structure or bipolar staining, but this is not sufficiently constant to be used as a diagnostic characteristic.

Staining.—Good with all the usual dyes. Old forms may stain badly and irregularly. Gram negative. Flagella stain easily by the Muir-Pitfield method.

Temperature.—Growth is good at any temperature between 22°C . and 37°C ., optimum probably being about 37°C ., although at 27°C . development is not much inferior. The thermal death-point is about 60°C . in ordinary bouillon, somewhat lower in presence of arsenite and at any reaction unfavourable to general growth.

Reaction.—Growth is best at about + 10 Fuller in bouillon, i.e. just on the alkaline side of true neutrality. It is restricted at neutrality to phenol phthalein, but is not inhibited until media become distinctly alkaline to that indicator, and neutral arsenate bouillon becomes alkaline by reduction. As its acid production from glucose shows, it can tolerate acidity as well as *B. coli*.

Oxygen Requirements.—The organism is facultative, but with a marked preference for aerobic conditions. Under strictly anaerobic conditions (Wright-Burri method) growth is comparatively feeble.

Growth in Bouillon.—Growth is very rapid, clouding being visible in less than 24 hours at 27°C ., and turbidity heavy in 48 hours. A few days later a heavy sediment begins to settle out, but the supernatant medium remains cloudy for long thereafter, and the sediment is incoherent and easily redistributed on shaking. The reaction becomes markedly alkaline to litmus and slightly alkaline to phenol phthalein. Initial growth is best at the surface, with a tendency to incoherent film formation. The odour in bouillon cultures is marked, but difficult to describe; a putrid fishy odour hardly distinctive enough to be of much service in picking out cultures from those of other putrefactive organisms. Behaviour in peptone water is similar to that in bouillon.

Indol Production.—Feeble or altogether absent in bouillon or peptone media.

H₂S Production.—In lead acetate bouillon, or Dunham's peptone medium there is moderate production of sulphuretted hydrogen. The reaction was not so marked as in the case of a strain of *B. coli* used for comparison.

Ammonia Assimilation and Ammonification.—In bouillon slight ammonification occurs, but with defective supply of organic nitrogen ammonium salts may be assimilated. Thus if a series of tubes be put up containing diluted hay decoction, growth after inoculation can be made much more abundant by the addition of glycerine and ammonium chloride, but not by the addition of glycerine alone. The assimilation of ammonia can be followed by formol titration.

Synthetic Media.—As remarked earlier, growth in synthetic media is poor, but can be made good by the addition of small amounts of organic matter of animal or vegetable origin. In Uchinsky's medium growth is at most very feeble.

Nitrate Bouillon.—Reduction to nitrate is very rapid, 0.25 per cent. NaNO_3 being practically completely reduced in forty-eight hours. There is, however, no gas formation, and since the strong sulphanilic reaction persists more or less indefinitely it is not likely that reduction proceeds further than the nitrite stage.

Glucose Bouillon.—Acid production is evidenced, but no gas formation. When the organism was first isolated acid production was slow, but after a few sub-inoculations into 2 per cent. glucose bouillon this function was much accelerated. Fresh cultures obtained by plating out from glucose bouillon manifested the same rapid acid production. The turning point to litmus in 2 per cent. glucose bouillon was reached within eighteen hours, and the turning point to methyl red ($P_{H.4}$ to $P_{H.5}$) within forty-eight hours, the titration +40 being then registered on the Fuller scale. The degree of acid production is therefore similar to that of *B. coli*, the distinction being the absence of gas formation.

Lactose Bouillon.—No acid and no gas. Inoculated from a culture which rapidly produces acid from glucose, a litmus lactose medium may go faintly acid within the first twenty-four hours, but thereafter goes alkaline.

Saccharose Bouillon.—No acid and no gas. The slight transient acidity shown with lactose is not shown with litmus saccharose bouillon and the medium goes slowly alkaline.

Glycerine Bouillon.—No acid and no gas.

Starch Bouillon.—Bouillon containing .05 per cent. of soluble starch showed no diminution of the starch-iodine reaction even after a fortnight. No distinct amylolytic or diastatic action could be detected.

Litmus Milk.—In the first twenty-four hours no change, or at most a faint transient reddening, is evidenced. By the second day slight alkalinity is noticeable, which slowly increases in the ensuing few days. There is little or no peptonization, however, and, apart from the slight alkalinity, no further change occurs even after the lapse of several weeks.

Nutrient Agar.—On ordinary bouillon-agar growth is rapid and punctiform colonies appear in two or three days, reaching a diameter

of anything up to three millimetres within a week—depending upon the crowding of the plate. Surface colonies are usually round, dirty white, flat or slightly raised, and sometimes with faintly iridescent borders. Microscopically the edges are entire or lobate, the general internal structure resembling that of *B. coli*. Subsurface colonies are smaller, denser, yellowish-brown, darker in the centre, usually lenticular in shape, and not infrequently develop a moruloid interior. If mixed with members of the colon-typhoid group it would be impossible to pick out the reducing organism by the macroscopic or microscopic appearance of its colonies. On .2 per cent. arsenite agar there would of course be no possibility of confusion with *B. coli*, since the latter is altogether suppressed, and the simplest way to separate the reducing organism from other members of the group to which it appears to belong is to plate out on arsenite agar.

The cultural similarity to coli extends to agar *slopes*, where the general appearance and rate of growth could not be distinguished from that of a strain of coli used for comparison. The slightly higher transparency of the growth and less noticeable darkening with advancing age were hardly distinctive enough for diagnostic purposes.

In *stab* culture growth is rapid and the track of the needle may be visible in twenty-four hours, developing evenly all the way down, filiform at first, but ultimately assuming a somewhat tuberculate character. The surface growth is round, flat, dirty white or grey, dull glistening, fairly firm, may reach a diameter of 1 cm. in a week, and finally spread to the edge of the tube. After keeping a few weeks a faint brownish tinge may appear. In glucose agar shake cultures surface growth is rapid, but small colonies develop without gas formation throughout the whole tube.

Gelatine.—On gelatine the growth is also coliform and no liquefaction occurs. On slopes or in stabs we could hardly distinguish the reducing organism from one of our laboratory strains of coli, although the incorporation of 0.1 per cent. to 0.2 per cent. of arsenite at once brought out the difference.

Potato.—On unwashed potato slopes growth is uncertain, but on well-washed slopes a fairly good growth is obtained, faint yellowish-brown after twenty-four hours and darkening slightly in the course of a few days. The potato itself takes on the brownish colour of the growth, which is flat, dull, firm, but soft and easily detached with a needle.

Pathogenicity.—In the ordinary sense it is non-pathogenic for rabbits and guinea-pigs. 2 c.c. quantities of bouillon cultures, twenty-four to forty-eight hours old, were injected intraperitoneally and subcutaneously without effect. It is quite possible, however, that under some circumstances mass cultures may be fatal in the same sense that *B. coli* may be.

Growth in Arsenical Media.—Growth on 0.2 per cent. arsenite agar is not quite so fast as on ordinary agar, but is otherwise very similar. Growth in 0.2 arsenite bouillon is just as rapid as in ordinary bouillon, and if successive sub-inoculations be practised into bouillon of steadily rising concentration of arsenite (at intervals of 0.1 per cent. As_2O_3) rapid growth can be maintained up to 0.8 per cent. As_2O_3 . Above this, inhibition begins to be marked, but we have secured fair growth in bouillon containing 1.2 per cent. As_2O_3 as sodium arsenite.

The reaction of course must be kept favourable by neutralizing the alkaline arsenite of soda used.

In arsenate bouillon growth is accompanied by reduction, but since reduction is accompanied by alkalization it never proceeds very far. In ordinary bouillon containing .2 per cent. As_2O_3 as *arsenate* reduction may only proceed to .1 per cent. As_2O_3 as *arsenite*, but in presence of glucose acid production more than balances the change in H^+ ion concentration due to formation of arsenite, and reduction may then be complete. It may be added that reduction of arsenate in presence of litmus is accompanied by more or less complete bleaching of the blue colour. The real upper limit of reduction is not far off the upper limit of tolerance for arsenite, provided other conditions of growth do not interfere. Metabolic products of growth, other than alkaline arsenite on one side and acid on the other, may possibly interfere with activity.

It was mentioned earlier that in the glycerine ammonium chloride mineral mixture reduction might proceed from zero to 0.2 per cent. arsenite within forty-eight hours, but that, no matter how much arsenate was present, further reduction proceeded only slowly to 0.3 per cent. and then stopped. At first we were disposed to regard this as a characteristic of the originally isolated strain and to consider that 0.3 per cent. represented its limit of tolerance. This, however, is not the case; unless we assume that the reducing faculty of the organism can be raised with extraordinary ease. In a mineral mixture (K_2HPO_4 , etc.) containing 1 per cent. of peptone, 2 per cent. of glucose, and 0.4 per cent. of arsenate, complete reduction to arsenite can be accomplished in two or three days. On addition of a further .2 per cent. of arsenate, reduction continues up to 0.6 per cent. As_2O_3 as arsenite. Still further addition of arsenate may or may not elicit any response according to the stage of exhaustion of the medium, but if sub-inoculation be then made into a similar fresh medium containing 0.6 per cent. of arsenite, reduction can proceed (more slowly) in spite of the initially high concentration of arsenite, and rise to above 0.8 per cent. in three days and to about 1 per cent. As_2O_3 (as arsenite) in a week. We attribute the originally noted lower reducing capacity to unsuitability of the synthetic medium used.

HYDROGEN ION LIMITS FOR REDUCTION.

By using the method of differential titration to a series of indicators of known H^+ ion turning point it was found that reduction could proceed both in acid and in alkaline media, the most favourable reaction being just on the alkaline side of neutrality, and the inhibiting limits of reaction being about P_H . 4.5 on the acid side and a little beyond P_H . 10 on the alkaline side. In the first series of tests a dilute dung decoction mineral mixture containing .2 per cent. K_2HPO_4 , .3 per cent. NH_4Cl , .1 per cent. As_2O_3 as arsenite, .3 per cent. As_2O_3 as arsenate, and 1 per cent. of glycerine, was used—200 c.c. quantities in flat bolt-head culture flasks. After sterilization sterile acid or alkali was added in amount, calculated by titration, required to bring the media to orange-red to methyl orange, orange-yellow to methyl orange, red-orange to methyl red, orange-yellow to methyl red, green-yellow to paranitrophenol, rose to neutral red, orange to neutral red, yellow

to neutral red, but colourless to phenol phthalein, faint rose to phenol phthalein, distinct rose-red to phenol phthalein, faint blue to thymol phthalein. The flasks then represented a series of H⁺ ion concentration ranging from P_H. 3 to P_H. 11. At each end of the series no growth was observed and no reduction took place. Twenty-four hours after inoculation the medium originally brought rose-orange to neutral red showed distinct superiority of growth over the media on each side of it. In two days the concentration of arsenite had risen from 0.1 per cent. to 0.19 per cent., while the flask assessed at P_H. 6 had only risen to 0.13 per cent., and that starting at about P_H. 8 had reached 0.16 per cent. Thereafter clear differences were obscured by the fact that as growth and reduction proceeded the reaction steadily changed in the direction of alkalinity owing to the transformation of neutral arsenate into alkaline arsenite. There could be no question, however, of the general trend of the results. A degree of acidity which gave a distinct red with methyl red (P_H. 4) allowed of no growth at all. Distinct orange to methyl red (P_H. 5) permitted initial slow growth and reduction and subsequent rapid growth and reduction as the reaction approached or passed the neutral point. On the alkaline side good growth was evidenced up to the turning point to phenol phthalein (P_H. 8), and fairly good growth and reduction for some considerable distance beyond it. Not until the reaction showed marked red to phenol phthalein, approaching faint blue to thymol phthalein, was reduction seriously inhibited. Although reduction is good on either side of true neutrality (to neutral red) slight alkalinity is preferred. Reduction in slightly acid, and in alkaline media, may be demonstrated very simply by using 1 per cent. peptone mineral mixture (K₂HPO₄ acting as buffer) with glucose or glycerine according as acid or alkaline reaction is to be encouraged. After inoculation into the glucose medium the turning point to methyl red may be reached in twenty-four hours. If now sufficient sterile neutral arsenate solution be added to bring the medium to .2 per cent. complete reduction may be effected in another forty-eight hours without any very material change in reaction—the acid production from glucose more than sufficing to counteract the alkali formation accompanying reduction. On addition of further arsenate further reduction may occur in the still acid medium, or if acid production is ended the reaction may pass over to alkalinity to neutral red. If glycerine be used in place of glucose the medium goes alkaline from the beginning, and with each successive addition of neutral arsenate reduction continues with rising alkalinity. Reduction still proceeds vigorously after distinct rose to phenol phthalein is registered. The bacterial activity in any medium and at any stage can at once be stopped by adding sufficient acid to turn methyl orange or sufficient alkali to turn thymol phthalein. A medium initially just too alkaline for growth may, however, on exposure to air change sufficiently to allow of reduction, presumably by absorption of carbon dioxide from the atmosphere.

Source.—Apart from its widespread occurrence in arsenical dipping tanks the reducing organism has been detected in the faeces of cattle. Indeed its origin is probably faecal, and although we have not yet tested its distribution in any great detail we have little doubt that the initial infection of a tank is brought about by faecal ejecta of dipped stock.

A number of other arsenic resistant organisms have been isolated from faeces and dipping tanks, but none of these have proved capable of reducing arsenate to arsenite, so that the probabilities are in favour of the view that only one reducing organism, and not several as at first conjectured, need be considered as of practical importance. The miscellaneous arsenic-resistant flora of dipping tanks will be dealt with at a later date.

Classification.—According to the system adopted by the American Society of Bacteriologists the organism would have the group number 222.233:3033. Its general cultural, biochemical, and morphological characteristics bring it into the *typhosus* group, although its high tolerance for arsenite and its outstanding capacity for reducing arsenate to arsenite differentiate it at once from most (if not all) other members of the colon-typhoid group. We have tested three strains of *B. coli* and one strain each of *B. enteriditis* (Gärtner), *B. para-typhosus* A, *B. para-typhosus* B, *B. typhosus*,* and *B. faecalis alcaligenes* in respect to tolerance for arsenite. In every case .05 per cent. As_2O_3 as arsenite, added to ordinary bouillon, was sufficient to retard growth, and 0.1 per cent. to inhibit development altogether. Arsenate was more readily tolerated than arsenite, but reduction of arsenate either did not occur at all or was at best feeble, and in any case limited to the degree of tolerance. In this series we have representatives of the main sub-divisions of the colon-typhoid group, so that the behaviour of the organism described here may be regarded as sufficiently unique to entitle it to a separate place in the family and to a name—*B. arsenreducens*. It, of course, any other member of the family—already described under another name—is subsequently found to possess this same faculty of reducing arsenate and tolerating high concentrations of arsenite, the name suggested here will have to fall away, but in the meantime a useful purpose is served by treating the organism as distinctive. The question as to how far other members of the group, such as *B. typhosus*, could be *educated* up to a high tolerance for arsenite is a matter for future investigation. Personally we are inclined to think that high tolerance may be an acquired characteristic, although from the behaviour of the particular organism under consideration it is apparent that the characteristic is more or less fixed once it is acquired, and is not easily lost again by sub-cultivation for several generations in arsenic-free media. Appearance and disappearance of particular characteristics under laboratory cultivation is of course now admitted. An interesting recently investigated case in point is provided by Ten Broeck † in his paper on “A non-gas-producing strain of hogcholera bacillus isolated from an old laboratory culture.” Some bacteria have an initial high tolerance for arsenite, and with them it is easy to raise the inhibiting concentration considerably by sub-cultivation into media of gradually increasing arsenical strength. *B. pyocyaneus* is an example.

* For *B. enteriditis*, *para-typhosus* A. and *B. typhosus* itself, and one strain of *B. coli* we are indebted to Dr. Watkins-Pitchford, Director of the Medical Research Institute at Johannesburg, to whom we wish to take this opportunity of tendering thanks. For *B. alcaligenes* we have to thank Mr. Montgomery, Veterinary Pathologist in British East Africa.

† C. Ten Broeck, *Journal Exptl. Medicine*, Sept., 1916. Vol. xxiv, No. 3.

APPENDIX.

A number of experiments have been carried out with mixed cultures of the reducing organism and the oxidizing organism. It is proposed to extend this work to include behaviour in presence of organisms which neither oxidize nor reduce but are merely arsenite-resistant, and the protocols of the work already done may therefore be held over for the moment. A brief statement of results is, however, of immediate interest, and it may therefore be stated that in presence of both organisms the chemical changes are dominated by the composition of the medium. If the medium is highly attenuated in regard to organic matter the oxidizing organisms gain the upper hand and oxidation of arsenite to arsenate takes place; not so much in virtue of numerical superiority as in virtue of greater biochemical activity. If the attenuated medium be then enriched by the addition of organic matter such as glucose, glycerine, fresh dung, etc., the reducing organisms rapidly outmultiply the oxidizing organisms and, in virtue of their numbers, effect reduction of arsenate to arsenite. The reducing organism grows much more rapidly than the oxidizing organism, but only reduces vigorously in presence of fairly large amounts of suitable organic matter. A given number of oxidizing organisms can oxidize arsenite very much more readily than the same number of reducing organisms can reduce arsenate.

Restriction of air supply—as in deep-layer cultures—favors reduction indirectly by limiting the operations of the oxidizing organism. The extent of oxidation on the one side and reduction on the other may also be limited by the extent of bufferization of the medium in which they are growing.

The practical bearing of these observations is that in tanks under field conditions the extent of oxidation is suggested as being largely controlled by the *frequency of use* of the dip. So far as field observations have gone the behaviour of dipping tanks is in line with the behaviour indicated in laboratory experiments. So long as the tanks are in regular use and fresh excreta are continually passing in, the reducing organisms appear to remain in the ascendant, and under the normal tank conditions of limited aeration no arsenate appears. The oxidizing organisms are nevertheless always there, and it is only necessary to expose a sample of the dip in shallow layer in the laboratory to effect oxidation. Under the more favourable conditions of aeration (and temperature) the dip soon becomes exhausted as a medium for reduction, and the superior biochemical activity of the oxidizing organisms then makes itself manifest. Oxidation may then set in and in a few days be complete, the rate depending upon the amount of faecal debris present. But the change can be promptly arrested, or reversed to reduction, by the simple addition of fresh faeces. Sometimes, as the observations of Lewis indicated, the amount of organic matter in the dip may be such as to allow of oxidization in shallow layer but reduction in deep layer.

In every tank we have examined, the presence of both oxidizing and reducing organisms could be demonstrated by sub-inoculation into differential media. But as a rule both are outnumbered by arsenic-resistant organisms of other types. The numerical distribution of flora under varying conditions has not yet been worked out.

In the Onderstepoort tanks, which are usually in use twice a week with different batches of animals, oxidation is never manifested in practice. If for any reason one tank falls idle oxidation may set in after a few weeks disuse. In regularly used municipal tanks we have never detected noteworthy oxidation, an observation in accord with the experience of other workers (Williams, Lewis) in South Africa. In privately owned tanks, especially where the number of stock dipped is small and the interval between dippings longer, oxidation appears to be of more common occurrence, although even then it is not so much to be feared as was formerly supposed.

In one case a tank came under observation where the stock were passed through a freshly prepared dip *once* and then remained undipped for a month. At the end of this time the unused tank showed marked oxidation. Dipping was then resumed at weekly intervals, and in the course of a few weeks the arsenate had disappeared. Thereafter the tank remained fully reduced. In this case the small amount of excreta entering the large volume of tank fluid during the first dipping had apparently given rise to a medium admirably suited for oxidation but not for reduction. When regular dipping was subsequently instituted the proportion of faecal matter was raised to such an extent that the dip was converted into a medium suitable for reduction and the earlier formed arsenate was reconverted to arsenite.

The moral of observations up to date is therefore very simple: *keep the tank in regular use.*

Chapin,* by extensive observation of the behaviour of tanks in the field, but without isolation or study of the bacteria concerned, has arrived at the same conclusion.

SUMMARY.

An organism which reduces sodium arsenate to sodium arsenite with great rapidity, and is capable of tolerating high concentrations of arsenite, has been isolated from an arsenical dipping tank. Its original source is probably faecal. It is the organism responsible for counteracting the oxidation which may be brought about by *B. arsenoxydans* (already described by us) and so far as is known at present it is the only one of practical importance in this direction. It is a vigorously motile non-sporulating organism, usually with four to eight flagella, but on occasion it may lose its motility and be cultivated for two or three generations as a non-motile bacterium. Its morphology, cultural behaviour, and general biochemical characteristics bring it into the typhosus sub-division of the colon-typhoid group. According to the classification system of the American Society of Bacteriologists it would have the group number 222.2333033, the facultative character not being very pronounced and a marked preference for aerobic conditions being shown. Since no other member of the colon-typhoid group which we have had an opportunity of examining displays either the degree of resistance to arsenite or the marked ability to reduce arsenate, shown by this organism, it is considered

* Chapin: "Studies on Changes in the Degree of Oxidation of Arsenic in Arsenical Dipping-baths." Bulletin of U. S. Dept. of Agriculture, No. 259, July, 1915.

that provisional christening is justified, and the name *B. arsenreducens* is suggested. The organism is rod-shaped, with rounded ends, and somewhat variable in size. Dimensions may range from 1μ to 6μ in length and 0.3μ to 0.6μ in breadth, the typical form being about 2μ by 0.4μ . Tendency to chain formation is marked. It stains well with all ordinary dyes and is gram-negative. Apart from the characteristics indicated by the group number it sometimes inclines more to the *coli* end of its group, as in sulphuretted hydrogen production, type and vigour of growth on potato and other solid media, and sometimes inclines more to the typhosus end as in absence of indol production and behaviour in milk media, and we should not be surprised if a strain were obtained in which acid production was lacking. Such a strain would merely represent a shifting of the characteristics further away from the *coli* end of the group towards the *alcaligenes* end.

In the ordinary sense it is non-pathogenic for rabbits and guinea-pigs; probably also for man.

Its outstanding characteristic is its high tolerance for arsenite and its capacity to reduce arsenate very rapidly in presence of sufficient organic matter. This it can do in both acid and alkaline media, a neutral or faintly alkaline reaction being most favourable. The reaction limits for growth are roughly assessed as lying between P_H . 4.5 and P_H . 10.5. The actual extent of reduction of arsenate varies with the composition of the medium and with its buffer effect; is greater, for instance, in glucose bouillon than in ordinary bouillon. The tolerance for arsenite in bouillon corresponds to about 1.2 per cent. As_2O_3 , although perceptible retardation in growth is noticeable below 0.8 per cent. Reduction can proceed almost to the limit of tolerance for arsenite provided no other limiting factors are at work.

The influence of the composition of the medium upon reduction is discussed briefly in relation to reduction and oxidation under practical conditions in dipping tanks in the field.

Notes on the Species of *Gastrophilus* found in South Africa.

BY

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IN introducing the present paper little need be said, except that the main objects in writing it are to place on record the species of *Gastrophilus* found to be parasitic upon horses, mules, and donkeys in their larval stages in South Africa, their life-histories, and the injurious effects which they have upon their hosts in this country.

Of the three species found, *Gastrophilus intestinalis* (equi) is the commonest, but both *G. pecorum*, Fabr., and *G. nasalis*, Linné, are far from being uncommon.

The larvae of the three above-mentioned flies are extremely common in the stomachs of horses in this country. The reason for this, we think, is most probably due to the dryness of the climate, especially as Velu* has observed that the long drought of 1913 in Morocco greatly favoured the parasites of many domestic animals, and that the larvae of Oestrids—*G. intestinalis*, *G. nasalis*, and *G. haemorrhoidalis*—were exceptionally common in horses.

The only other fly belonging to the family Oestridae, which has been found to be parasitic upon horses in South Africa, is a "nasal fly," which I take to belong to the genus *Rhinostrus*. The larvae have only been found once in the nasal cavity of a horse at Onderstepoort, and are exceptionally rare, as this is the only instance we know of it having been found in this country.

The flies belonging to the genus *Gastrophilus* are popularly known as "Bot Flies," and their larvae as "bots." In South Africa, however, the larvae are usually known as "papias."

They are large, hairy flies, with rather robust bodies and large heads. The mouth is atrophied, the antennae are extremely short, and consist of three joints with an arista, which is also three-jointed, and situated on the dorsal surface of the third joint near the anterior margin. The arista, however, shows much variation in some of the specimens of *G. intestinalis* in the laboratory collection; these will be fully dealt with later on. The compound eyes are comparatively large and are separated in both sexes; between the compound eyes there are three ocelli. The thorax has a distinct transverse suture. Wings of the muscid type; the fourth longitudinal vein runs straight towards the border of the wing; squamae small. The females can be distinguished from the males by the elongate ovipositor, which is bent

* Velu : Recueil Med. Vet. Alfort, XCII, No. 13, pp. 408-410 (1916).

under the abdomen of the fly when at rest. In the males there are a pair of black hooks or clasping organs at the apex of the last abdominal segment. In their larval stage they are parasitic in the stomach, pharynx, oesophagus, and rectum of equines.

The genus contains about fourteen species, of which three are known to occur in South Africa, namely, *G. intestinalis*, De Geer, *G. pecorum*, Fabr., and *G. nasalis*, Linné.

There is another species which has been found in German East Africa, also in the Sudan, namely *G. asinus*, Brauer, which is in all probability only a variety of *intestinalis*. Although this species has not up to the present time been found in South Africa it is quite possible that it will be imported into this country with remounts returning from East Africa at the conclusion of the campaign. This species very closely resembles *G. intestinalis*, De Geer, in general appearance, but can be distinguished by the following characters: The hair on the underside of the front femora is black, stiff, and straight, instead of yellow, silky, and curved at the ends as in *G. intestinalis*, De Geer; also the transverse band on the wing is more sharply defined, extending back from the fifth and sixth vein without coming into contact with the hind margin.

Both the adults and larvae of the species to be found in South Africa can be easily identified by the following tables:—

TABLE OF ADULTS (AFTER GERMAR).

1. Wings with brown bands.....	2
Wings hyaline.....	3
2. Discoidal cell open.....	<i>G. intestinalis</i> , De Geer.
Discoidal cell closed.....	<i>G. pecorum</i> , Fabr.
3. Anterior basal cell nearly, or quite, equal to the discoidal cell in length.....	<i>G. nasalis</i> , Linné.
Anterior basal cell markedly shorter than the discoidal cell.....	<i>G. haemorrhoidalis</i> , Linné. *

TABLE OF LARVAE. †

1. Each segment of larvae with two rows of spines.....	2
Each segment with only one row.....	<i>G. nasalis</i> , Linné.
2. Dorsal surface with two complete rows of spines on the anterior borders of the segments 2-8 and a few at the sides of the 9th.....	3
Dorsal surface with two complete rows of spines on the anterior borders of the segments 2-5; on the 6th there is a bare space in the middle, which is more marked on the 7th, greater on the 8th, and the 9th usually unarmed. Colour blood-red.....	<i>G. pecorum</i> , Fabr.
3. Spines of the first row of each segment decidedly larger than those of the second. Colour usually yellow with a reddish tinge.....	<i>G. intestinalis</i> , De Geer.
Spines of the first row of each segment scarcely so large as those of the second. Colour blood-red.....	<i>G. haemorrhoidalis</i> , Linné.

* This species, popularly known as the red-tailed bot fly, has not up to the present time been found in South Africa, but I have included it in the tables, as I think it quite possible that it may be imported into this country with horses coming from Europe or America. In general appearance the adult fly closely resembles that of *G. nasalis*.

† This table will also serve to differentiate the pupae.

(1) *G. INTESTINALIS*. DE GEER (1776).*Synonyms*:—*Oestrus bovis*, Linné (1761).*Gastrophilus equi*, Clark (1797).*G. equi*, Leach (1817).*O. gastricus major*, Schwab (1840).*G. bengalensis*, Macq.

Although the name given by Clark to this common bot fly of the horse is the one which is usually adopted in all the textbooks and literature on these flies, it will be seen from the above synonymy that De Geer had instituted the name of *intestinalis* for this species no less than twenty-one years before Clark named it *equi*. Therefore, in accordance with the rules of nomenclature the former name should have priority over the latter.

Habitat.—This species, which is widely distributed throughout the world, is exceptionally common in South Africa, it being almost impossible to make a post-mortem upon a horse without finding the larvae in the stomach.

Hosts.—The larvae are found in the stomach of the horse, ass, and mule, particularly the former.

The laboratory collection also possesses some larvae, which I take to belong to this species, taken from the stomach of a zebra in Zululand by Mr. D. T. Mitchell.

The larvae have also been recorded as having been found in the stomach of the dog, but cases are by no means common. The dog cannot be considered a normal host, and it is very doubtful whether the larvae could live for any length of time in the stomach of a carnivorous animal. I think there can be no doubt that the larvae were introduced into the stomach with horse droppings, which dogs are often in the habit of eating.

DESCRIPTIONS OF THE DIFFERENT STAGES.

The *ova* are attached to the hairs of the host by means of two parallel flanges, which encircle the hair and extend from the posterior extremity for about half the length of the ovum. They are conical in shape, yellow in colour, and are provided with an operculum at their apices, which is more or less flat above and convex below. They are transversely striated throughout, except the operculum, which is smooth. Length 1.25 mm.

The *larvae* on hatching are long and fusiform in shape with thirteen segments. They are white in colour with dark spines, and destitute of hairs. The first (the cephalic segment) has a lance-like mandible directed forwards, and a number of small movable spines which are situated on the anterior margin. Second segment with a number of spines on the anterior margin, those in front being the longer, and two rows of longer spines on the posterior margin. Spines are also present on posterior margins of segments 3-10, but segments 11-13 are destitute of spines. At the apex of the thirteenth segment there are two elongated tubercles, each of which has a small spiracle at its apex.

The larva after undergoing two moults has only eleven segments, which are more convex on the dorsal surface than on the ventral; it is conical in shape, being narrow at the base and broad at the apex.

The cephalic segment has a number of minute spines behind the antennae and buccal organs; there are also situated between the first and second segments two stigmata, which appear as small knobs or fissures, and are partly hidden in the folds of the skin. The segments 2-8 each have two complete rows of backward-projecting spines which are light brown at their base and dark at the apex. On the ninth there is an interruption in the middle of the dorsal surface, and on the tenth the dorsal surface is devoid of spines, except for one or two at the lateral margins. On the eleventh segment, posteriorly, there are stigmatic plates which contain two lateral gill-plates and a median stigmatal leaf, and which are protected by lip-like organs. The gill-plates consist of a coronour chitinous substance. Each plate is reniform or crescent-shaped and constituted by three concentric arches, each having a longitudinal groove on which open small apertures arranged in pairs. It is through these apertures that breathing takes place.

In colour the larvae vary somewhat, but as a rule they are either red, or yellow with a reddish tinge, usually turning yellow just before pupating. Length when mature 16-20 mm.

The Pupa.—The pupa-case is formed by the hardening of the skin of the larva; it is dark reddish-brown or black in colour. Length 15-16 mm.

The Adult.—*Head* yellow in colour, with a few dark brown or black hairs on the forehead; the face and jowls are slightly lighter and sparsely clothed with white pile; antennae yellowish-brown.

Thorax black, covered with reddish hairs, except behind the suture, where there is usually a transverse band of black hairs; on the suture there are two small conspicuous admedian tufts of reddish-yellow hairs. Scutellum reddish-brown, covered with longish upright reddish hairs.

Abdomen reddish-brown with rows of black spots on the posterior borders of the segments; spots of the same colour are also often present on the anterior borders.

Legs yellowish-brown; femora clothed with yellow pile.

Wings transparent, each with a dark transverse band, and two spots of the same colour at their apices.

Length of female (excluding ovipositor) 12-15 mm.; of male 12-13 mm.

In the laboratory collection there are, in addition to a large number of specimens in which the arista are normal, eight females and eleven males, in which the arista has become greatly modified and enlarged. At first I was led to believe that I had found a new species, but last year I succeeded in obtaining more of these interesting forms, and found that in three of the specimens the arista showed marked variations; not only did this apply to the arista of the different specimens, but also to those of the same individuals. Also, the shape of the segments and the number and position of the hairs in the majority of the remainder of the specimens also show very slight variations.

The following are the descriptions of the arista of three males:—

Specimen No. 1 (page 646, fig. 14).—The right arista is composed of three segments, and a large leaf-like appendage and a claw at its apex. The first two segments are equal in length; the second is

narrower at the base than at the apex, and the third segment is slightly more than three times as long as the second segment, is slightly broader at the apex than at the base, and has several short bristles, a row of longish hairs on the outer margin and four long hairs at the apex. The large leaf-like appendage at the apex of the third joint resembles the petal of a lily in shape. The large claw, which is situated beneath the leaf-like appendage, is brown in colour and black at the apex, and resembles the unguis of the feet. The left arista differs from that of the right, in that the first and second joints are fused together, the apex of which is twice as broad as the apex of the second joint of the right arista. The third joint is slightly longer and very much broader; also the number and position of some of the hairs and bristles on the third segment are somewhat different.

Specimen No. 2 (page 647, fig. 15).—The right arista is two-jointed with a leaf-like appendage and a claw at the apex. The leaf-like appendage is smaller than those in specimen No. 1. The claw has been omitted in the figure as it is completely hidden by the leaf-like appendage when the antenna is viewed from above. The first and second joints are fused together, and are about equal in length and breadth to the first joint of the right arista of specimen No. 1. The third joint is slightly shorter and narrower than the third joint of the right arista of Specimen No. 1, and has only two hairs in the middle on the dorsal surface, also two short bristles and two long hairs at the apex. The arista of the left antenna consists of a single joint, which is long and hair-like, except at the apex, where it becomes swollen, and terminates in two pointed lobes; on the dorsal surface, near the base, there is a longish hair.

Specimen No. 3 (page 647, fig. 16).—The arista of the right antenna is three-jointed. The first two segments are about equal in length and breadth to those of the normal arista; the third segment, however, is shorter and thicker than that in a normal specimen, and has a short bristle on the inner side, near the middle.

The arista of the left antenna is also three-jointed, but of an entirely different shape. The first segment is very slightly broader than that of the right arista; the second much shorter. The third segment is nearly as broad as the second at its base; it then widens out for one-third of its total length; then it becomes abruptly narrow and continues as a hair as far as the apex, which is slightly swollen. In the middle of the swollen portion, on the inner side, there is a spine and a bristle, the former being about twice as long as the latter. From the above description and figures on pages 646-647 it will be clearly seen that the arista of the antennae are of two distinct kinds with intermediate forms. One, the normal form (which we will hereafter call *type A*), is three-jointed with the third segment long and hair-like and destitute of hairs. The other, the right arista of specimen No. 1 (which we will call *type B*), is three-jointed, with a leaf-like appendage and a large claw-like spine at the apex; the third segment is considerably shorter and broader than in *type A*, and has several hairs and minute spines on the surface. Now the question arises, can we consider the specimens with the *B type* of arista as belonging to a distinct species? There can be absolutely no doubt that the differences are of specific importance, but as the arista of the fifteen specimens of *type B* in the collection vary slightly I have preferred to include them for the time being as abnormal forms of *G. intestinalis*. I

think, however, that there can be no doubt that a new species is being formed.

Of the three males, of which the arista are figured, I regard the left arista of specimen No. 1 as abnormal, and those of specimens Nos. 2 and 3 as the result of crossing between specimens of the A and B types.

THE LIFE-HISTORY.

In the Transvaal the adult flies are found from January to May, but are most numerous during the months of February to April. The female, when she is about to lay her eggs, hovers near an animal on which she intends to deposit them with her body bent downwards and the ovipositor thrust forward and fully extended. When an egg is ready to be deposited she darts towards the animal and fixes it to the hair in an instant, and then retreats again until she is ready to deposit another one. This operation is repeated at very short intervals, so that a large number of ova may be deposited upon an animal in a comparatively short time. The female lays about 400 to 500 ova, all of which may be placed under favourable conditions upon the same animal. They are usually fastened to the hairs of the forelegs, neck, shoulder, chest, or belly, and sometimes even to the mane and other parts of the body. They are often laid close together and can be readily detected; also the empty egg capsules, which often remain attached to the hairs for some considerable time after the larvae have escaped.

The length of time the eggs take to hatch varies somewhat. Bracy-Clark, the celebrated English veterinary surgeon, who was the first to work out the life-history—he having published his results in 1796—wrote that the eggs took twenty-five to thirty days to hatch, whilst Joly asserted that they hatched in four to five days. Osborn, who made a careful series of observations on the ova in America, which he carried out during the summer months of 1893-4, was, from the results of his experiments, able to draw up the following conclusions: (1) That the eggs do not hatch, except by the assistance of the horse's tongue. (2) That the eggs appear to be fully developed three or four days after being laid, but that hatching only occurs with difficulty before the tenth day and most readily after the fourteenth day; that they lose their vitality after a period varying between the twenty-eighth day and fortieth day, the bulk not surviving for more than four weeks.

The following experiment was carried out at this laboratory in order to ascertain the length of time the ova take to hatch in this country:—A horse, which was free from ova, was let loose in a field and examined every evening until a female had laid ova on it. It was then transferred to a stable and examined twice a day to see if any of the ova hatched. The result was that all the ova hatched in seven to thirteen days, the majority hatching on the thirteenth day.

It will be seen that the results obtained from this experiment tend to show that the egg stage does not last quite so long in South Africa as what it does in North America. The young larva, which is able to escape from the ova through the operculum being broken off by the moisture and friction caused by the horse licking itself or its companions, attaches itself to the tongue or lips of its host, and is in this way taken into the mouth and thence finds its way into the stomach, where it attaches itself to the lining of the mucous membrane by

means of the two buccal hooks. They live for about ten or eleven months in the stomach of their host, during which time they undergo three moults. They are usually found attached to the cardiac portion of the stomach and occasionally in the fundus and duodenum. During the months of December to May, when the larvae become fully mature, they detach themselves from the mucous membrane, are carried through the intestines, and finally leave their host with the faeces, when they at once commence to burrow into the ground and pupate. The pupa stage lasts from eighteen to twenty-five days, at the end of which time the fly breaks off the operculum and escapes. The larvae, however, which leave their hosts during the month of May pupate, but always fail to hatch. The flies only live for a short period, during which time they are unable to nourish themselves as their mouths are entirely rudimentary. They are very rapid fliers and are not often met with, except the females, when they are busy laying their eggs. They may, however, occasionally be met with resting on blades of grass. It is best, therefore, in order to obtain the adult flies, to collect the larvae and breed them out.

There is a robber-fly (*Hoplistromenus serripes*, Fabr.), found in South Africa, which very closely resembles *G. intestinalis* in general appearance when at rest, and could easily be mistaken for the bot-fly by any one who was unacquainted with it. It is very common in the Transvaal, and may often be seen resting on roads, horse manure, or on blades of grass. It is chiefly parasitic upon small dung beetles. It can, however, be at once distinguished by the hind femora, which are dilated, and by the antennae, which are large and of a different shape.

(2) *GASTROPHILUS PECORUM*, FABR.

Habitat.—This species, which is found throughout Europe, was very common at Onderstepoort during 1913, and again in 1916. Larvae of this species were also forwarded to the laboratory by Major Cannon, S.A.V.C., who collected them from the pharynx of horses at Luderitzbucht, South-West Africa, and Kajiado, British East Africa. The horses in question were all imported from the Union.

Host.—The larvae are found in the stomach, pharynx, and oesophagus of horses.

DESCRIPTIONS OF THE DIFFERENT STAGES.

The *ova* are said to resemble those of *G. haemorrhoidalis*.

Numerous unfertilized ova, which were laid on corks of test-tubes by females which had been bred out, were all black in colour and resembled the ova of *G. intestinalis* in shape. They differ from them, however, in not having flanges, instead of which they have a very short stalk at their posterior extremity. Under a microscope this stalk is seen to be composed of very fine minute fibres, which are stuck to the surface on which the eggs are laid. Also, they appear to be smooth and shiny and not striated as in *G. intestinalis*. They are usually placed vertically, or nearly so, to the surface on which they are laid, but are occasionally found at almost any angle. Length .86 mm.

The *larvae* resemble those of *G. intestinalis*, and can only be distinguished by the absence of spines in the middle of the dorsal surface of segments 6 to 8 and the entire absence of spines on the ninth

segment; also by the colour, which is blood-red. Length when mature 14-16 mm.

The *pupa* is reddish-brown in colour. Length 13-15 mm.

The Adult.—Forehead brown, sparsely clothed with yellow hairs; face and jowls covered with longish hairs of the same colour. Antennae reddish-brown. Eyes of the male set closer together than what they are in the female.

Thorax.—Dark brown or black, clothed with golden or coppery-coloured hairs, which are more numerous round the margin. In the male there is a transverse line of black hairs immediately behind the suture; scutellum covered with longish upright golden hairs and a few black ones.

Abdomen of the female black, with the first two segments sparsely clothed with yellow hairs, the remaining segments sparsely clothed with either yellow or black hairs. In the male the abdomen is brown, covered with copper-yellow hairs; a few black ones are also sometimes present on the two apical segments.

Legs reddish-brown. *Wings* slightly fuscous, with a dark, transverse band, and a large apical spot of the same colour.

Length of female 13 to 15 mm.; of male 11 to 13 mm.

THE LIFE-HISTORY.

The life-cycle is practically the same as in *G. intestinalis*, except for the following differences:—

The adult flies appear from February to April.

The larvae are usually found in the pharynx, cardiac, and fundus, or more rarely in the duodenum, the oesophagus, pharynx, or attached to the epiglottis. Those which are attached to the pharynx, oesophagus, or epiglottis are often deeply embedded in the mucosa and submucosa. According to Neumann they remain temporarily attached to the mucous membrane of the rectum before leaving their host to undergo their nymphosis.

(3) *GASTROPHILUS NASALIS*, LINN. (1761).

Synonyms—

Oestrus orterinus, Clark (1797).

O. salutiferus, Clark (1815).

O. clarkii, Leach (1817).

Gastrus salutaris, Meigen (1824).

O. duodenalis, Schwab (1840).

This species is popularly known as the "Chin Fly."

Habitat.—The fly is very common in South Africa. It is also found throughout Europe, North America, and in parts of Asia.

Host.—The larvae are found in the duodenum of the horse.

Description of the Different Stages.

The *ova* (fig. 1) are elongated-oval in shape, yellowish-white in colour, and the two parallel flanges extend nearly the whole length of

the ovum, commencing just in front of the posterior extremity, and continuing to just behind the operculum, which is dome-shaped and much narrower than in the ovum of *G. intestinalis*. They are transversely striated, except the operculum, which is unsculptured. Length 1.25 mm. The larva on hatching has only twelve segments. They are long and slender, being only slightly broader in the middle than at the extremities. They are white in colour and have several regular rows of hairs on segments II-XII, which can be distinctly seen in figure 1. The cephalic segment has paired mandibles directed forwards, and a number of minute spines on the anterior margin. Segment II with similar spines on the posterior margin. Segments III-X with an irregular row of long, narrow spines on the posterior margin. The apex of segment XII does not terminate in two long tubercles as in the *G. intestinalis*—the spiracles hardly projecting beyond the extremity.

The larvae (third stage) differ from those of the two preceding species by the presence of only one row of spines on the anterior borders of each of the abdominal segments, excluding the apical segment. They are blood-red in colour. Length 13 to 16 mm.

The pupae are dark-brown or black in colour. Length 12 to 15 mm.

The adults are more hairy than either of the two preceding species.

Head, dark reddish-brown; forehead, face, and jowls covered with yellowish-brown pile; antennae ferruginous.

Thorax, black, covered with orange or reddish-yellow hairs, except behind the suture, where there is a transverse row of black hairs, and on the posterior margin, on each side of the scutellum, where the hairs are white; there are also a few white hairs usually present on the lateral margins in front of the wings. On the suture there are two admedian tufts of reddish-yellow hairs. Scutellum dark brown, covered with erect reddish-yellow hairs.

Abdomen black, covered with fine hairs, which vary slightly in colour; they are always white on the second segment, black on the third in the female, and either black or orange in the male; on the succeeding segments they are white in the female and orange in the male.

Legs: the femora are dark brown, or almost black, with longish curved yellow and white hairs, which are more numerous on the anterior and mid femora; tibiae dark brown, and tarsi reddish-brown.

Wings: hyaline.

Length of female (excluding ovipositor) 10 to 12 mm.; of male 12 to 13 mm.

THE LIFE-HISTORY.

The life-history differs from that of *G. intestinalis* only in the following points:—

In Natal the adult flies are found from October to February, whereas in the Transvaal they appear during the month of December to the beginning of May.*

* Since the above was written, a male hatched at Onderstepoort on the 5th June, 1918.

The females lay their eggs on the hairs round the lower lip and those on the intermaxillary space between the mandibles, and have also been reported to have laid their eggs in the nostrils of their host.

The ova, unlike those of *G. intestinalis*, do not require friction and moisture in order for them to hatch, as the young larvae are able to break off the operculum without any assistance. Ova which were collected from horses, and kept in tubes at room temperature, hatched in a few days, whereas those of *G. intestinalis* when kept under similar conditions always failed to hatch, even after several months.

The larvae are only found in the duodenum, near the pylorus.

The pupa stage lasts from 18 to 55 days in the Transvaal, but from 18 to 25 days is the usual period, and from 22 to 40 days in Natal.

THE EFFECTS OF THE LARVAE UPON THEIR HOSTS.

With regard to the effect that these larvae may have upon the health of their hosts, very divergent opinions have been given by the different authorities. Some affirm that they perforate the walls of the stomach and cause death, whilst others—of whom we may cite Bracy-Clark—affirm to the contrary. The latter were of an opinion that the larvae had a beneficial influence upon the economy of their hosts, in serving natural stimulants to the digestive functions. It was due to this belief that Meigen and Clark designated the names *G. salutaris* and *O. salutiferus* for *G. nasalis*, in order to signify the beneficial effects the larvae were supposed to have on the digestive functions.

It is not to be wondered at that some of the early authorities were led to believe that the larvae were beneficial to their hosts, when we come to consider that it is almost impossible to make a post-mortem on a horse without finding some larvae in the stomach, how numerous the larvae frequently are in the cardiac and duodenum, and the entire absence of symptoms—which could be attributed to the presence of larvae in the stomach—shown by the horses during life.

There can be no doubt whatever that the larvae are of no beneficial importance to their hosts, but, at the same time, the injurious effects they have frequently been supposed to have upon their hosts have often been highly exaggerated. This is due to the fact that horses frequently die showing no external or internal symptoms which can be attributed to the cause of death, and the result is that the larvae which are found in the stomach are often held responsible for the death of their hosts.

It is exceptionally common amongst the farmers of this country to attribute the death of their horses to *Gastrophilus* larvae—even when they only find a few in the duodenum or cardiac—when they are unable to discover the actual cause of death.

1. The effects of the larvae in the duodenum, cardiac, and fundus.

The number of larvae that may be found collectively in these portions of a stomach may vary from three or four to several hundred. Velu, in making autopsies upon horses in Morocco during the long drought of 1913, usually found more than 1000 larvae in their

stomachs. But cases of such large numbers of larvae being found in the stomach of a horse are very exceptional, and the frequency of such numbers in the above cases was entirely due to the drought, which favoured their increase. They are found in the stomach throughout the year, but are less numerous during the latter part of December to April.

In order to give the reader some idea as to the prevalence of the larvae of the different species occurring in horses in South Africa, the following record is given:—

Out of 340 horses which died at Onderstepoort from 1st November, 1916, to 1st November, 1917, larvae of *G. pecorum* were found in the pharynx on four occasions, and in the oesophagus on ten occasions; larvae of *G. intestinalis* were found in the cardiac portion on one hundred and thirty-three occasions, and those of *G. pecorum* on forty-five occasions; larvae of *G. nasalis* were found in the duodenum on ninety-five occasions, those of *G. intestinalis* on six occasions, and those of *G. pecorum* on four occasions; and larvae of *G. intestinalis* were found in the fundus on fifty-four occasions, and those of *G. pecorum* on twenty-six occasions.

They cannot be considered as seriously affecting the health of their host in any way unless, as seldom happens, they burrow into the mucous membrane and perforate the walls of the stomach, in which case the horse would, in all probability, die. It is, however, possible that, if an animal is in an extremely poor condition, and badly infected with bots, they may have serious effects upon the health of their host.

From October, 1908, to October, 1917, no less than 5015 autopsies were made on horses at Onderstepoort, and of that number only two showed perforation of the mucous membrane of the stomach, due to the larvae of *Gastrophilus intestinalis*.

The following is the post-mortem record of one of the horses whose stomachs were perforated by the larvae:—

Horse No. 10623. *Date of death*, 11th October, 1916.

Condition poor. *Rigor mortis*: not present. *Integument*: abrasion of left orbit. *Visible mucous membrane*: pale. *Natural openings*: normal. *Flesh*: pale. *Subcutaneous tissue*: very little fat present. *Salivary glands*: submaxillary pale. *Lymphatic glands*: the bronchial and mediastinal were enlarged, and showed marked hyperaemia; mandibulars moist; pharyngeals and upper cervicals moist; consistency normal. *Tongue*: muscle pale. *Oesophagus* normal. *Larynx* and *pharynx* pale. *Cervical trachea*: mucous membrane pale, containing white froth and mucous material. *Thoracic trachea*: containing froth and reddish-yellow mucus.

Left lung: partly inflated; posterior portion of main lobe showing a raised area, firm in consistency; size 17 by 10 cm. *Pleura* in periphery opaque, and raised above the foci. Anterior lower border of main lobe consolidated. The covering pleura smooth, tissue underneath showed whitish areas. The tissue was firm on section, uniformly consolidated, and contained white pus. The left anterior lobe was firm, and showed cavities containing a creamy pus. Surrounding tissues also contained pus. *Right Lung*: the posterior portion of the main lobe showed a raised area. The pleura above it was smooth,

and showed ecchymoses. On section it showed a white opaque area, irregularly outlined, but distinctly marked off. The remainder of the lung showed gelatinous infiltration, and was consolidated as described. Upper border with similar conditions. The middle lobe the same as the left lobe.

Heart: the right ventricle contained coagulated blood. The ostium admitted four fingers. The left ventricle was the same. The fatty portion of the epicard was replaced by gelatinous substance. The sulci of both ventricles showed petechiae. The longitudinal sulcus of the left ventricle showed slight ecchymoses in patches. The right endocardium showed ecchymoses below the valves. The valves showed slight gelatinous infiltration. The left endocardium and valves were normal. *Myocardium*: left 2 cm.; right 1.25 cm. On section it was seen to be slightly paler in colour. The consistency was normal. *Liver*: capsula normal; on section the acini were found to be distinct; colour and consistence normal. *Pancreas*: pale. *Spleen*: the spleen measured 31 by 13 by 2 c.m. The capsula was normal; on section it was seen to be dark; consistence normal. *Suprarenal Glands*: normal. *Kidneys*: the capsula of the left kidney was easily detached; the malpighian bodies were distinct; colour pale; consistence normal. Right kidney as in the left.

Stomach: the fundus was normal. The cardiac portion contained numerous larvae of *G. intestinalis*, several of which had perforated the mucous membrane in places. (See page 643, fig. 6.)

Small intestines pale. *Large intestines*: caecum and colon slate coloured. *Rectum* normal. *Bladder*: the bladder contained yellow, turbid urine. *Brain* normal. *Bone marrow*: the humerus and femur showed marked haemorrhagic infiltration. *Pathological Anatomical Diagnosis*: ecchymoses of the epicardium and right endocardium; bronchiectasis, broncho-pneumonia in both lungs; perforation of the mucous membrane of the stomach by *G. intestinalis*.

Cause of death: Broncho-pneumonia.

2. The effects of the larvae in the pharynx and attached to the epiglottis.

In South Africa it is not an uncommon occurrence to find the larvae of *G. pecorum* attached to the musosa of these parts, and often deeply embedded in the submucosa. As a rule they are not numerous, the number usually varying from one or two to twenty. Their presence in these situations often causes great irritation, and may even be responsible for death of their host.

The following is an extract from a report by Major Cannon, S.A.V.C., who has carefully recorded the symptoms of a horse infected with larvae in the pharynx, which was in his charge at the Base Veterinary Hospital, Luderitzbucht, South-West Africa:—

“On arrival at my depot the horse stood with his head near the ground, and a profuse flow of ropy saliva was coming from the mouth. breathing slightly increased. temperature normal. Examination of the throat and submaxillary revealed no swelling, and no obstruction could be found along the course of the oesophagus.

" Upon elevating the head for the purpose of making an examination of the mouth, the horse struck out and stamped with both fore feet, walked backwards, coughed violently, and fell on his side, with glottic spasms, threatening to bring almost immediate death. He rallied, however, and in a few seconds got on to his feet again, staggered about for a minute, and then came to a standstill, with legs straddled, his head extended in line with the neck, facial expression distressful, much champing of the jaws, rapid efforts at deglutition, clicking sounds were emitted, and the salivary evacuations enormously increased. I came to the conclusion that the horse had some foreign substance lodged in his throat.

" After allowing some twenty minutes quietude the mouth was cleaned as much as possible of mucous accumulations, and by leaving the head in a position indicating most comfort to the horse, examination was effected, without the presence of any foreign body being located. My interference, nevertheless, excited another fit of coughing, but with less alarming results. The mouth was flushed with a solution of ac. boric and alum, a mustard liniment and cotton wool pack applied around the throat, and belladonna and pot. chlor. electuary prescribed for the tongue.

" Several hours later the horse was standing quiet, but listless and depressed. The salivary flow from the mouth maintained. Food was refused, but water eagerly accepted. All attempts to swallow caused the water to return through the nostrils, and efforts were made to restrain coughing. The following day I found his condition the same. Any attempt to elevate the head was resented, and threatened to bring further paroxysms of coughing, so disturbance in this manner was carefully avoided, as it was noticed that the cough was less frequent and severe whilst the head was depressed. The temperature remained normal, no complications were presented, and no swelling of the throat became visible, although slight manipulation of this part stimulated deglutition, and threatened to excite coughing.

" At the end of the third day no appreciable improvement in the animal's condition was indicated, so I decided to dress the throat with a dilute solution of tinct. iodine and glycerine. This was accomplished by means of a mop made of cotton wool secured to the end of a stick. The applications caused the horse great distress, and, upon withdrawing the mop, discovered numerous larvae* of the *Oestrus* family adhering thereto. A second and third application later brought about similar results. On the fifth day a marked improvement was noticed. Salivary evacuations were much reduced, cough less painful, the head was restored to normal position, but food was refused until the next day, when an attempt was made to swallow some gruel, a considerable portion of which was returned through the nostrils.

" The mopping process was continued with success, and gradually, day by day, the powders of deglutition were much improved and the appetite restored.

" Necessarily, the horse had lost much in condition, but was ultimately fit for discharge in about thirty-four days from date of

* Some of these larvae, which were forwarded to the laboratory for identification proved to be *G. pecorum*.

admission to hospital. During this period I was favoured at intervals by the arrival of ten more horses, all presenting the same chain of symptoms already described, but in varying degrees of intensity. Having discovered the cause of their troubles to be the presence of *Oestrus* larvae involving the pharynx, the application of the mop and iodine treatment was promptly pursued, and the disappearance of alarming symptoms became immediately progressive. In every case food such as bran and grass was refused for some days, but water was always accepted and preferred to gruels.

"In no instance was a discharge from the nostrils observed, except when an attempt was made to swallow liquids. Not one of the eleven cases brought under treatment registered a temperature of higher than 102.2, and complications were not recorded. The cough in all cases was moist, and represented a series of expulsive efforts in rapid succession, the inspiratory efforts being, as it were, choked off."

In Europe the larvae of *G. haemorrhoidalis* sometimes attach themselves to the pharynx and epiglottis, and several instances of horses dying from asphyxia due to the presence of these larvae in the pharynx have been recorded, but I do not know of any case on record of the larvae of *G. pecorum* having been found in the pharynx of horses in Europe.

3. *The effects of the larvae in the oesophagus.*

The larvae of *G. pecorum* have been found attached to the mucous membrane and muscular wall of the oesophagus of horses at Onderstepoort on more than one occasion, but cases are extremely rare, and only a few larvae were found on each occasion.

Major Cannon forwarded some larvae of *G. pecorum* to the laboratory, which were collected from the oesophagus of a horse—which had been imported from South Africa—at the Remount Camp, Kajiado, British East Africa.

The following is an extract from his report:—

"The horse had pleurisy complications, and died on 17th August, 1916, when Major Montgomery undertook the post-mortem examination. Upon opening the thorax a quantity of water was liberated, the lungs were shrunken, and the pleura wholly with inflammatory deposits of a creamy white colour and adherent to the ribs, spleen much enlarged but firm, liver normal.

"The stomach contained a tablespoonful of sand, about a gallon of water, and numerous bots attached to the mucosa. The bowels contained only watery constituents, and, like the stomach, were free from inflammatory lesions. The larynx and pharynx were removed, the former normal, but sections of the latter revealed the presence of several clusters of *Oestrus* larvae attached (although many had been removed by previous dressing), with perforations and ulcerations clearly defined.

"The oesophagus contained over 70 larvae along its course, some deeply embedded in the muscular wall, others attached to the mucous membrane in clusters of six and eight together. There were numerous perforations, and an inflammation pervaded its course. The frontal

sinuses and nasal cavities were free from invasion, hence the absence of catarrhal symptoms always manifested.

"The pleurisy complication was viewed by me as the primary cause of death, but the oesophagus exhibited the presence of these parasites not only deeply embedded in the walls, but perforations right through the thoracic sections.

It may, therefore, be feasible to assume that the perforations were primarily the cause of the pleuritic lesions, and that the larvae, at certain stages of their development, are migratory. The establishment of hydrothorax would indicate that pleurisy had existed for some considerable time, but it is conceivable that the presence of an irritant giving rise to uncontrollable fits of coughing would aggravate a rapid development of any chest complication with fatal tendencies."

Owing to the fact that larvae which are found in the pharynx are often deeply embedded in the mucosa and submucosa, and that those which were found in the oesophagus of the horse must have been there for at least two to six months before the animal died, I am inclined to think that the perforations of the oesophagus were primarily caused by the larvae, and not by the pleuritic lesions.

Larvae which are found attached to the pharynx and oesophagus are usually very small or only half-grown, and I am, therefore, inclined to think that the majority which are found in these regions only live there during the first few months of their larval stage, and then migrate to the stomach.

4. *The effects of the larvae attached to the rectum.*

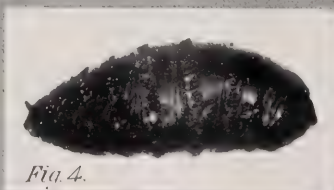
According to Neumann, the larvae of *G. haemorrhoidalis* and *G. pecorum* fix themselves to the mucous membrane of the rectum for some time before being evacuated, and that they have sometimes occasioned troubles that have not always been without gravity.

Hertwig reports having observed a horse which made such violent efforts to defecate, in consequence of the presence of some thirty larvae in the rectum and anus, that there occurred an eversion of the rectum, which required a surgical operation to reduce it.

Although the larvae of *G. pecorum* are commonly met with in horses at Onderstepoort when making autopsies, they have only been found in the rectum on one or two occasions, and I am inclined, therefore, to think that the majority, at least, do not attach themselves to the folds of the rectum before being evacuated.

EXPLANATION OF PLATES.

- Fig. 1.—Larva of *G. nasalis* escaping from egg. $\times 40$.
 Fig. 2.—Larva of *G. nasalis* just after it had hatched. $\times 40$.
 Fig. 3.—Full-grown larva of *G. intestinalis*, De Geer. $\times 3$.
 Fig. 4.—Pupa of *G. intestinalis*. $\times 3$.
 Fig. 5.—Larvae of *G. intestinalis* attached to a portion of the mucous membrane of the stomach.
 Fig. 6.—Shows perforations of the mucous membrane of the stomach, due to the larvae of *G. intestinalis*.
 Fig. 7.—*G. intestinalis* ♀. $\times 3$.
 Fig. 8.—*G. intestinalis* ♀, showing abnormal antennae. $\times 3$.
 Fig. 9.—*G. nasalis* ♀. $\times 3$.
 Fig. 10.—*G. pecorum*, Fabr. ♀. $\times 3$.
 Fig. 11.—*G. pecorum*, Fabr. ♂. $\times 3$.
 Fig. 12.—Larvae of *G. pecorum*, Fabr., attached to the pharynx of a horse.
 Fig. 13.—Lateral view of a normal antenna of *G. intestinalis*.
 Fig. 14.—Dorsal view of the aristae and basal portions of the third joints of the abnormal antennae of specimen No. 1.
 Fig. 15.—Same of specimen No. 2.
 Fig. 16.—Same of specimen No. 3.
 Fig. 17.—Larva of *G. intestinalis* escaping from egg. $\times 40$.
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*Fig. 1.**Fig. 2**Fig. 3.**Fig. 4.**Fig. 5.**Fig. 6.*

*Fig. 7**Fig. 8.**Fig. 9.*

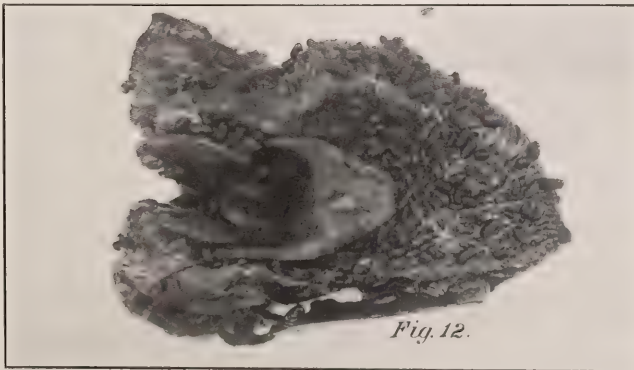
*Fig. 11.**Fig. 10.**Fig. 12.*



FIG. 13.



FIG. 14.



FIG. 15.



FIG. 16.



FIG. 17.

Notes on the Intoxication by Gastrophilus Larvae.

BY

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Veterinary Research Officer, Onderstepoort.

Notes on the Intoxication by *Gastrophilus* Larvae.

By G. v. D. W. DE KOCK, M.R.C.V.S., Veterinary Research Officer,
Onderstepoort.

INTRODUCTION.

THE subject of the intoxications produced by animal parasites is still in a very confused state, and we are quite in the dark as to how much of the effects they produce is merely mechanical or is due to soluble toxins that they may secrete or excrete.

Humphrey (1887) produced death in two guinea-pigs by the intraperitoneal injection of 6 c.c. of hydatid fluid. In case of the dog, inoculated with 60 c.c., no death resulted, but symptoms of dyspnoea, tachycardia, and hypotension. Viron (1892) killed guinea-pigs by the subcutaneous inoculation of a substance that he prepared by saturating echinococcus fluid with ammonia sulphate and precipitating it with 95 per cent. alcohol. Laveran and Mesnil (1899), on the other hand, inoculated rabbits and guinea-pigs with large doses of echinococcus fluid from cysts obtained from sheep, without producing any symptoms of an intoxication. G. Guerrini (1911) came to the conclusion that the injection of the nucleo-proteids of the cestodes in no way gave rise to specific symptoms, and in no case were there any symptoms of an intoxication. Joest (1906) did not observe in animals any acute symptoms either by subcutaneous, intraperitoneal, or intravenous injections of hydatid fluid. Boidin and Laroche (1910) killed guinea-pigs (240-310 grm.) by intraperitoneal inoculation with 50-100 c.c. of echinococcus fluid, which was compressed in vacuo to 1-10th its original bulk. The animals died in from twenty-four hours to four days after the inoculation.

According to V. Linstow there exists in the *Ascarides* a volatile substance which has a peculiar and unpleasant odour, and is very irritating to the mucous membranes. The author had the opportunity of testing this on himself after touching his eye with a finger that had been in contact with one of these worms, causing a conjunctivitis and chemosis which lasted some time. Goldschmidt (1910) gives a number of similar cases which occurred amongst some of his students who had been dissecting these worms. Some people seem to be more susceptible than others. Arthus and Chausson saw three persons who had worked with the *ascarides* from the horse and had contracted conjunctivitis and laryngitis. These authors also injected rabbits with a watery extract taken from living round worms. The animals died in about ten minutes after the injection of 2 c.c. subcutaneously. Weinberg and Julien (1911) observed a swelling of the eye with lachrymation, in 145 horses out of 220 treated, after the instillation

into the eye of sterile "peri-enterique" fluid of the ascaris. In some horses even more acute symptoms such as those of diarrhoea, dyspnoea, and profuse sweating were observed. They lay stress on the fact that this fluid should be prepared in a sterile and pure state as described by them in their researches. Dobernecker (1912) states that the ascaris contains a toxin which is present in the body fluids. Animals injected with such a fluid die. He gives the toxic doses for white mice, guinea-pigs, and rabbits. S. P. Bedson (1913) from his observations made upon guinea-pigs injected with sterile "peri-enterique" fluid from *Ascaris megalocephala* and filtered extracts of various tape worms (*T. plicata*, *T. perfoliata*, *T. siganata*), drew the conclusion that the reaction of the experimental animal to the verminous toxin is specific. Alessandrini and Paolucci (1909) and Guerrini (1910) in their researches came to the conclusion that the ascarides fluids do not contain such toxins, but that death is due to the injection of such animals with fluids containing infectious germs.

According to Blanchard. *Dibothriocephalus latus* frequently caused Anaemia, which has been attributed to a poison liberated by the parasite when it undergoes disintegration and possibly as a secretion of the living worm. Schaumann and Tallquist (1898) found that extracts from these worms were toxic to dogs in whatever way they were administered and caused a marked anaemia.

Weinberg (1912) comes to the following conclusions: It is without a doubt that the helminths liberate substances that have a toxic action on the body of the host. These substances vary in the degree of their toxicity and in the manner of their action with the different parasites. The kind of injury depends not only on the number and localization of the parasites and the resorption of the toxins, but also on the susceptibility of the host.

Adami holds that the mere existence of toxic substances in the bodies of these animals is no evidence that they excrete toxins. It may, for instance, be pointed out that the blood of species of warm-blooded animals is toxic for other species; that is no proof that these secrete actively toxic substances

V. R. Seyderhelm and K. Seyderhelm (1914) come to the conclusion that pernicious anaemia can be artificially produced in horses by injecting watery extracts of *Gastrophilus equi* and *haemorrhoidalis* larvae, and that according to its action, physical and chemical behaviour, the active ingredient is an animal poison or toxin which was called "oestrin" by the authors. This toxic action is absolutely specific for the horse and mule. The *oestrin* is resorbed from the gastro-intestinal tract, and it is present in the material secreted by the *Gastrophilus* larvae. The cases of pernicious anaemia artificially produced with *Gastrophilus* larvae extracts, are caused in a similar manner, as they are by means of infected blood into susceptible horses. Also the blood of such infected horses (with larvae) can again transmit the disease to other horses. The pernicious anaemia of horses that occurs naturally is not caused by ultraviolet viruses but by the excreted *oestrin* of the *Gastrophilus* larvae. The toxic action of the sub-species, *Gastrophilus haemorrhoidalis*, is far greater than that of *Gastrophilus equi*.

Ries (1906) calls attention to the fact that he once made the statement that he accused the larvae of *Gastrophilus* of playing a part in the

propagation of pernicious anaemia, either by being the carrier of infection by opening up a port of entrance or by causing a general weakening of the power of resistance. Ries thinks that the frequency of the disease on isolated farms situated in the wooded regions, supports his theory. The Japanese Commission (1914) found that bot-flies do not belong to the blood-sucking insects, but since their larvae are found in the horse's stomach the virus may thus be taken from the sick horses and transmitted to the healthy. To exclude this possibility several larvae were taken out of the stomach of a horse dying of the disease, crushed in a mortar, filtered, and injected into healthy horses, but the result was negative. Furthermore, in 1912 bot-flies were exterminated from all horses before pasturing, and it was noticed that the removal of these insects did not have an influence on the prevalence of the disease. Carré and Vallée (April, 1916) published a further article on the etiology of equine anaemia, mainly devoting attention to the recent work of Seyderhelm and Seyderhelm on this subject. They admit the existence in the horse of a verminous anaemia, gastrophilic or strongylic, but they refuse to admit the identity of equine pernicious anaemia with that of verminous origin. They consider it proved by their own researches and the numerous confirmations that they have received from all quarters, including South Africa (*vide* Third and Fourth Annual Reports of the Director of Veterinary Research), that in the horse there exists an infectious anaemia of inoculable microbial nature due to a filtrable virus. They also observed in their researches, acute symptoms of intoxication by injecting watery extracts (of three larvae) intra-jugularly. They further showed that pernicious anaemia virus is destroyed when subjected to a temperature of 60° C., whereas the oestrin toxin is not destroyed at that temperature. Ries (1916) again publishes his views upon this question. He disagrees with the opinion of Carré and Vallée, and he finds support for his belief in the researches of Seyderhelm and Seyderhelm, and affirms that intoxication by *oestrin* is the cause of anaemia in horses in the districts in which he studied the disease. He cites the cases of such horses, the post-mortems of which all showed the existence of larvae of the *Gastrophilus* in the stomach, and in each horse there was a verminous aneurism of the anterior mesenteric artery. Ries believes that equine pernicious anaemia is a gastrophilic anaemia, and that the part played by aneurismal thrombosis should not be overlooked. In 1917 comes a report by Sohns and Soetedje from Netherlands East Indies establishing the presence of infectious pernicious anaemia in that country. The authors do not consider a gastrophilic spread of the disease possible in that country owing to the fact that the Bot Flies cannot exist there and were seen for the first time by Smit in an imported horse from China in 1917. A paper by Hadwen and Bruce, "Anaphylaxis in Cattle and Sheep produced by the Larvae of *Hypoderma* and *Oestrus*," appeared in 1917, and its conclusions can be briefly summarized as follows: (a) Anaphylaxis has been produced in cattle and sheep and small animals with extracts of the larval forms of *H. bovis*, *H. lineatum*, and *Oestrus ovis*; (b) the reaction can be induced by crushing and returning an extract of an animal's own larvae into the jugular, showing that larvae living in the animals make them sensitive; (c) natural cases of Anaphylaxis are described where no injection has been given and where injury had ruptured the larvae subcutaneously, liberating

their contents in sufficient quantities to produce shock; (d) the symptoms in acute Anaphylaxis were immediate, the first noticeable sign being an extremely tired look, succeeded almost immediately by salivation, tears, defaecation, then by signs of asphyxia, and death. In the chronic form the symptoms were a little less rapid and not so severe; in addition there were oedemas, especially of eyelids and anus, and marked irritation of the skin; (e) small animals were sensitized with warble extracts and showed signs of anaphylaxis following the second injection. The first positive results where anaphylactic phenomena were successfully produced in animals by hydatid fluid from man were those of Chauffand, Boidin, and Laroche (1909). Similar results were obtained later by Puntoni (1910), Ghedini, and Zamorani (1910) and Weinberg; the latter obtained fatal anaphylaxis in 10 to 30 per cent. of the animals actively sensitized.

This is a very brief summary of the divergent opinions existent: (a) About the intoxication produced experimentally in animals by extracts of some of the nematodes, *Gastrophilus*, *Hypoderma*, and *Oestrus* larvae; (b) the rôle played by the *Gastrophilus* larvae in the causation of equine pernicious anaemia. The present paper was chiefly undertaken to prove or disprove such a "parasitic" etiology of pernicious anaemia, and it was during its study that symptoms of intoxication by *Gastrophilus* larvae were observed. The paper is divided into two parts:—

1. Intoxication produced in animals by *Gastrophilus* larvae;
2. Transmission of pernicious anaemia in horses by means of *Gastrophilus* larvae extracts.

Technique.

The *Gastrophilus* larvae from horses and donkeys were collected at post-mortems; they were separated from ingesta, etc., by washing well in tap-water, placed in a flask with some clean tap-water, and kept in an icebox, or in cold storage. There the larvae were left for one, two, or more days, as the case might be, until they were required for experiment. Watery extracts were used for most of the experiments, prepared as follows: The larvae selected were pounded up in a mortar into a pulp, to this was added physiological water usually in the proportion of 1:20, the whole was thoroughly mixed and then passed through muslin. The filtrate so obtained, designated *watery extract*, was opaque, and varied in colour from a light dirty grey to a dirty brick-red, and was used for the experiments. In some of the experiments the watery extract was further passed through filter paper and then through a Berkefeld filter. In such experiments (a) *watery extract* would be the pounded-up larvae and water passed through muslin; (b) *filtrate* would be the mixture obtained when such a watery extract is passed through a Berkefeld filter.

The DOB (serial) number of the horse from which the *Gastrophilus* larvae were taken, and the date of its death, are given in brackets. In some experiments the history of the horse is given. The horses utilized for the different experiments belonged to two classes, viz., (a) horses immunized with one or more horse-sickness viruses, and which could not be utilized for the preparation of anti-horse-sickness serum, either on account of their condition, or through being too small for that purpose; (b) horses regarded as susceptible to horse-sickness

which were used in cases of experiments where attempts were made to transmit horse-sickness by means of the watery extracts of *Gastrophilus* larvae. Injections were either carried out subcutaneously or intravenously in the large animals, and subcutaneously, intraperitoneally, intravenously, or subdurally in the small animals. The time was noted, as far as possible, when symptoms, etc., were observed.

1.—INTOXICATION BY *GASTROPHILUS* LARVAE.

Experiment No. 1.—To ascertain whether watery extracts from *Gastrophilus* larvae collected at post-mortems from the stomach of horses could produce symptoms of an intoxication when injected intrajugularly into other horses.

(16th March, 1915): *Horse* 9063, injected intrajugularly with 60 c.c. watery extract from 30 *Gastrophilus* larvae (*horse* 8722, 14th November, 1915) in physiological water. The larvae were kept overnight in an icebox.

Some minutes after the injection the horse showed symptoms of uneasiness, laboured respiration, passing of small quantity of faeces, respiration increasing in frequency, and distinctly jerky movements of the abdomen. Serous discharge from nostrils in drops first, increasing to a small stream, followed by the discharge of yellow gelatinous-looking flakes. Breathing through the mouth followed (inspiratory). Nostrils widely open; perspiration increased, hair wet, weakness of muscles increased; pupils dilated; drops down and dies almost at once. Time elapsed from injection until death was twenty to twenty-five minutes.

The chief post-mortem lesions were: Lobar pneumonia; ecchymoses of both endocardiums; thrombosis of pulmonary arteries, and branches of anterior mesenteric arteries; nematode-worm infection of anterior mesenteric artery and large intestines; some gastrus larvae were present in stomach.

(20th March, 1915): *Horse* 8969, gelding, condition rather poor, aged. (11.25 a.m.): Injected intrajugularly with 20 c.c. watery extract of 60 *Gastrophilus* larvae (*horse* 8722, 14th November, 1915), in 200 c.c. physiological water, kept in the icebox forty-eight hours after mincing up.

(11.26 a.m.): Animal shows uneasiness; lifts its tail; laboured and jerky respiration, frequency 52; chewing movements, saliva escapes in strings from the mouth; tears running from the eyes, and watery discharge in the form of drops from both nostrils. (11.28.): Straining movements of the abdomen; a few balls of faeces passed; pulse imperceptible. (11.30 a.m.): Profuse perspiration; the animal assumes a position as if wanting to defecate, straining; respiration more jerky. Rocking movements of the body; saliva still escaping in strings from the mouth; conjunctival mucous membrane injected and pupils dilated. Mucosa of lips and gums bluish in colour. (11.33 a.m.): Respiration 44; still standing in the same position; sweat running from the body. Extremities cold; pulse imperceptible; gasping; expression of the face anxious and shows distress; shakes the head; body temperature cold; rectal temperature 100.8°; yellow froth from the mouth. (11.37 a.m.): Goes down and lies in sternal position; turns over on to the side, followed by symptoms of suffocation and death.

The chief post-mortem lesions were: Hyperaemia of right lung; slight ecchymoses of left endocardium, some gastrus larvae present in the stomach.

Conclusions.—Two horses injected intrajugularly with the watery extract of *Gastrophilus* larvae produced per-acute symptoms of an intoxication, and death within half an hour.

Experiment No. 2.—To ascertain the largest quantity of watery extract of *Gastrophilus* larvae that could be given to a horse (*a*) intrajugularly, (*b*) subcutaneously, (*c*) by drenching, without causing the death of the animal.

(a) *Intrajugularly*.—(26th July, 1915): Horse 8673, bay gelding, condition moderately poor. (11.30 a.m.): Injected intrajugularly with the watery extract of one *Gastrophilus* larva (weight 200 mg.) (horse 9330, 22nd July, 1915), in 20 c.c. physiological water.

(11.37 a.m.): Animal restless; moves in a circle; front limbs lifted alternately. (11.38 a.m.): Chewing movements; yawning; licking movements; tail raised. (11.40 a.m.): Quivering of the muscles of the hindquarters noticed; off hind limb raised and the tail switched. (11.41 a.m.): Quivering becomes more marked. (11.42 a.m.): Hind limbs spread apart; first flatus, then dry faeces, passed. (11.43 a.m.): Symptoms resembling those of colic shown, e.g. straining, head and neck stretched forwards and downwards. (11.44 a.m.): Skin damp, but body temperature good; perspiration on the inside of the thighs in the form of streamlets running down the leg; lachrymation; watery discharge from both nostrils. (11.46 a.m.): Small jets of urine passed; discharge from the nostrils like streamlets. (11.47 a.m.): Hind limbs extending backwards; straining; a few balls of faeces passed, followed by a grunt. (11.48 a.m.): Animal staggers; perspiration round the anus. (11.50 a.m.): Goes down and lies spread out; several times regains the sternal position, but immediately falls back on to its side again. (11.52 a.m.): Rises with difficulty, especially noticed in the hindquarters, but almost immediately goes down again on to its side; several times it regains the sternal position, only to drop back on to the side. (11.53 a.m.): Breathing markedly laboured and jerky, tail extended; anus open, forcing out balls of faeces and straining. (11.54 a.m.): Nostrils open; respiration 33 and abdominal; conjunctiva slightly cyanotic; pulse imperceptible. (11.57 a.m.): Eyes closed; nostrils open; breathing is stertorous; animal now remains lying stretched out on its side. (11.58 a.m.): Comatose; eructation of gas every now and then. (11.59 a.m.): Hind parts cold; coat clammy. (12.0 p.m.): Inspiratory dyspnoea marked; breathing with mouth open. (12.1 p.m.): Tongue hanging out, quite limp; grunting; head extended upwards and backwards from the ground every now and then. (12.4 p.m.): Grunts; the mouth is opened with each inspiration; ears drawn backwards. (12.5 p.m.): Regains sternal position, but only for a second, and then drops back with force on to the ground. (12.6 p.m.): Twitching of the eyes; marked and laboured inspiratory dyspnoea; head and neck extended backwards and jaw drawn downwards. (12.9 p.m.): Eye reflex still present. (12.11 p.m.): Animal dead.

The chief post-mortem lesions were: Ecchymoses of both endocardiums and epicardium; *Gastrophilus* larvae present in stomach; ecchymoses in bronchi. Sclerostomes present in large intestines.

(28th July, 1915): Horse 9328, bay gelding, condition poor. (11.15 a.m.): Injected intrajugularly with 1 c.c. of the watery extract of one *Gastrophilus* larva (weight 400 mg.) (horse 8673, 26th July, 1915) in 20 c.c. physiological water.

(11.25 a.m.): Licking movements of the lips; lifting the tail and passing a small quantity of dry faeces. (11.27 a.m.): Licking movements more frequent. (11.28 a.m.): Snorting and looking at its off-side; licking movements; yawning; shaking of head. (11.31 a.m.): Yawning and looking at near-side. (11.34 a.m.): Licking movements; pulse, respiration, and temperature up to now normal. (11.40 a.m.): Appears quite calm and attentive. (1.0 p.m.): Animal normal. This animal was temperatured daily, and kept under observation until the 24th September, 1915, i.e. for nearly two months, and no clinical observations were recorded against it.

30th July, 1915): Horse 9048, bay gelding, condition moderately poor. (11.58 a.m.): Injected intrajugularly with 10 c.c. of the watery extract of one *Gastrophilus* larva (500 mg.) (horse 9418, 30th July, 1915) in 20 c.c. physiological water.

(11.59 a.m.): Licking movements. (12.0 p.m.): Blowing its nose; somewhat restless. (12.1 p.m.): Shifting its legs, spreading them out as if wanting to stale, and shaking its head and body. (12.2 p.m.): Defaecating and lifting the tail; shaking the head several times and swishing the tail. (12.3 p.m.): Frequently looks at the near-side; shakes the head and smells the ground. (12.4 p.m.): Defaecating; lifting the tail; smelling the side; licking movements.

(12.5 p.m.): Legs spread out and animal defaecates; the faeces passed up to now have been of a somewhat softer consistence, but of a normal colour; tail kept lifted; pawing and rubbing the nose against side of the shoulder; looking at its sides and shaking its head. (12.6 p.m.): Blowing the nose and rubbing it against the pole; respiration 68; swishing of the tail. (12.9 p.m.): Passing a good quantity of faeces; smelling the limbs; tail lifted; nostrils moving and shaking of head. (12.13 p.m.): Looking at its sides; yawning; small drops of a watery discharge noticed coming from the nose; shifting the legs about and swishing the tail. (12.15 p.m.): Ears drawn back; serous discharge from the nose more profuse. (12.19 p.m.): In the region of the nose, and on the inside of the thigh, the animal sweats; respiration 70; nostrils moving and ears drawn back. (12.23 p.m.): Temperature 101.4°; appears quieter, resting its hind limbs alternately; discharge still present from the nose. (12.27 p.m.): Shifting the legs about; looking at off-side; shaking the head and pawing with the off foreleg. (12.30 p.m.): Yawns and looks at off-side; this is repeated several times. (12.35 p.m.): Attempting to lie down. (12.41 p.m.): Coat quite damp; slight quivering of muscles in the region of the flank; shaking the head, looking at the side and smelling the ground. (12.43 p.m.): Several times attempted to lie down; kicking at the abdomen; looking at off-side and then at near-side. (12.50 p.m.): Brought to a loose box and there noticed to pass a small quantity of watery faeces; walking round in the box, blowing the nose; going down and lying in the sternal position, it rolls over to the side, then stands up again and shakes the body. (12.55 p.m.): Down again, lying on the side, stretched out; assumes sternal position; rises and shakes the body. (12.58 p.m.): Lying stretched out and rolling; good quantity of flatus and small quantity of watery faeces passed; quivering of muscles in the region of the flank. (1.0 p.m.): Lying spread out and rolling; then assumes the sternal position; this is repeated several times. (1.40 p.m.): Sternal position; dyspnoea; quivering of muscles in the flank; rises, shaking the head; passing a fair quantity of watery faeces, with an offensive odour; observed to chew the bedding; walking round in its box, then again lying spread out. (3.0 p.m.): Horse found standing; appears quiet, feeding, and seems to have recovered. (6.0 p.m.): Animal feeding and appears to have recovered completely. Horse 9048 was temperatured daily and kept under observation until the 23rd September, 1915, when it was discharged from experiment. No clinical observations were recorded during that time.

Conclusions.—In three horses injected intrajugularly with a watery extract of one *Gastrophilus* larva and 20 c.c. physiological water:—(1) Horse 8673 died of acute intoxication in about 45 minutes when given the full dose; (2) horse 9328 showed very slight symptoms of an intoxication, lasting about ten minutes, when given about one-twentieth of a full dose; (3) horse 9048 showed acute symptoms of intoxication, which lasted about three hours, when given 10 c.c. of the mixture, i.e. about half the full dose.

(b) *Subcutaneously.*—(28th July, 1915): Horse 9095, grey mare, condition moderately poor. (12.17 p.m.): Injected subcutaneously with watery extract of one *Gastrophilus* larva (horse 9330, 22nd July, 1915) and 20 c.c. physiological water.

(12.25 p.m.): Lifting the tail, and passing a small quantity of normal faeces. (12.27 p.m.): Grinding of the teeth. (12.45 p.m.): Passing a quantity of urine, normal in colour and consistence. (12.55 p.m.): Pulse 45; respiration 11. (1.10 p.m.): A slight swelling had developed at the seat of inoculation, about the size of the palm of one's hand, somewhat hot and painful. (28th July, 1915): Swelling increased in size and fluctuating. (30th July, 1915): Abscess which had formed had burst, discharging a dirty yellow, very putrid smelling material. (3rd August, 1915): Swelling and discharge much less. (9th August, 1915): Small wound left at the seat of inoculation; no discharge present. (19th August, 1915): Animal had fallen off in condition slightly. It was kept on temperature and under observation until 23rd September, 1915. It only showed a slight reaction with remittent fever from forty-ninth to fifty-fourth day; maximum temperature 103° on the forty-ninth day. No other clinical symptoms were recorded.

(26th July, 1915): *Horse* 8971, condition moderately poor. (2.14 p.m.): Injected subcutaneously with the watery extract of 20 *Gastrophilus* larvae (horse 9330, 22nd July, 1915) in 20 c.c. physiological water.

(2.16 p.m.): Pawing with off fore; lifting the tail; restless. (2.20 p.m.): Yawning and looking at its side. (2.23 p.m.): Standing with hindlegs extended backwards; licking movements. (2.24 p.m.): Yawning repeatedly. (2.25 p.m.): Licking movements; raising the tail. (2.27 p.m.): Passing a quantity of normal faeces and looking at its near-side. (2.31 p.m.): Rubbing its nose against the fore limbs. (2.33 p.m.): Spreading out its legs as if wanting to stale and finally passing a dirty, light greenish urine; tail remaining lifted. (2.34 p.m.): Slight champing movements of the jaws; restless; rubbing its nose; licking movements; shaking the head and body. (2.40 p.m.): Moving from side to side and shaking the head. (2.45 p.m.): White foam present on the margins of the lips. (2.47 p.m.): Lifting tail and defaecating; stringy saliva escaping from the mouth. (3.5 p.m.): Salivation more copious. (3.6 p.m.): Passing a few balls of faeces. (3.8 p.m.): Defaecating; faeces soft; kicking at belly; rubbing head against off front limb and then against the door; watery faeces passed. (3.10 p.m.): Again flatus and watery faeces passed; pawing; rubbing nose against front limbs; shaking the head; respiration increased. (3.17 p.m.): Kicking at belly, and looking round at side; shaking the head. (3.20 p.m.): Salivation in form of white strings still dropping from the mouth; looking at near-side; lifting hind limbs alternately and standing for a short while with legs spread out. (3.30 p.m.): Respiration further increased and more laboured; slight quivering of muscles in the region of the flank; shaking the head; looking at near-side; head extended forwards and lowered; very restless; kicking at the abdomen alternately with hind limbs. (3.36 p.m.): Making attempts to lie down; nostrils dilated. (3.40 p.m.): Wet on inside of thighs from profuse sweating; head and neck lowered. (3.45 p.m.): Perspiration in the form of drops running from head and quarters; very restless; passes a small quantity of watery faeces. (4.0 p.m.): Again watery faeces—with an offensive odour—passed; lips swollen and commissures of mouth beginning to swell; eyes kept half-closed. (4.10 p.m.): White foamy mucous discharges from nostrils; again defaecates; tail raised. (4.20 p.m.): Defaecates; respiration 36 and abdominal. (4.25 p.m.): Animal grows quieter; pulse 60; less discharge from mouth and nostrils. (4.35 p.m.): Making attempts to feed. (4.45 p.m.): Lying in sternal position, somewhat dull and half-sleepy in appearance. (6.0 p.m.): Standing and feeding; swelling of lips, etc., still present, but slightly decreased. (8.0 p.m.): Animal seems to have recovered completely. (27th July, 1915): A fairly firm, hot, and painful swelling at seat of inoculation; swelling of lips, etc., has subsided. (28th July, 1915): Swelling still firm, but with a slight tendency to point. (30th July, 1915): Swelling has burst; a very disagreeable, dirty white discharge escaping through a small wound. (9th August, 1915): Small wound still present at the seat of inoculation; most of the swelling has disappeared. This animal was temperatured daily and kept under observation until 23rd September, 1915, and no other clinical symptoms were recorded until the animal was discharged from experiment.

(28th July, 1915): *Horse* 9019, bay gelding, condition moderate. (11.45 a.m.): Injected subcutaneously with watery extract of 40 *Gastrophilus* larvae (weight 13.5 gm.) (horse 8673, 26th July, 1915) in 20 c.c. physiological water.

(11.48 a.m.): Shaking the head; licking movements; swishing of tail; smelling the ground, and restless. (11.49 a.m.): Lifting the legs alternately; shaking the head and body; looking at the off-side repeatedly. (11.57 a.m.): Yawning; licking movements. (12.2 p.m.): Lifting the tail; spreading out the legs as if wanting to stale; taking a few seconds before it passes some urine, which is of normal colour and consistence. (12.6 p.m.): Swishing the tail and shaking the body slightly. (12.13 p.m.): Shaking the body and rubbing the nose against the pole. (12.20 p.m.): Lifting the tail and passing a fair quantity of faeces. (2.5 p.m.): Rather a painful swelling, about the size of a hand, has developed at the seat of inoculation. Animal quiet; respiration slightly increased; pulse 48. (3.0 p.m.): Animal seems to have recovered; feeding. (30th July, 1915): Swelling has burst, a disagreeable discharge is escaping from a small wound. (9th August, 1915): No discharge from wound. (19th August, 1915): Wound nearly healed; near hind cannon slightly swollen.

Animal temperatured daily and kept under observation until 23rd September, 1915, and no other clinical observations were recorded until the animal was discharged from experiment.

(6th August, 1915): *Horse* 8825, bay gelding, condition moderately poor. (12.7 p.m.): Injected subcutaneously with watery extract of 60 *Gastrophilus* larvae (weight 24 grm.) (horse 9410, 6th August, 1915).

(12.8 p.m.): Licking movements. (12.10 p.m.): Looking at off-side twice; shaking the head; chewing movements. (12.13 p.m.): Chewing movements rather frequent; restless; changing and lifting the position of the legs frequently. (12.17 p.m.): Lifting the tail and defaecating. (12.20 p.m.): Slight quivering of muscles of forequarters; very restless; lifting the tail and kicking at the abdomen several times; chewing movements. (12.22 p.m.): Sweating noticed on the nose and anterior portion of the body which is quite wet from perspiration; kicking at the belly; lifting tail and passing quantity of normal faeces whilst grunting, pawing, and shaking the head. (12.23 p.m.): Drops of perspiration from perineal region, inside of thighs, nose, front and hind limbs; respiration increased; body temperature good; animal very restless, moving from side to side. (12.28 p.m.): Blowing the nose several times; head, breast quarters, and inside of thighs quite wet; pulse increased in frequency and small; lifting tail and defaecating. (12.33 p.m.): Drops of perspiration from the body more frequent; shaking of head; lifting the tail and passing flatus; conjunctival mucous membrane slightly cyanotic. (12.35 p.m.): Lifting tail and defaecating; head lowered; shaking head and blowing nose; perspiration still more profuse; chewing movements; pulse imperceptible. (12.45 p.m.): Lifting tail and defaecating; shaking the head; respiration increased, laboured and abdominal. (12.50 p.m.): Placed in a loose-box; shaking head rather violently; picking up some of the bedding and chewing it; passing a quantity of faeces, which is partly watery; rubbing the head against the manger; further attempts at feeding; standing quieter; drops of perspiration still rolling down. (2.0 p.m.): Perspiration much less; the coat in places still quite wet; respiration still slightly abdominal; some of the food supplied at 1 p.m. had been eaten. (3.0 p.m.): Animal seems to have recovered. (6.0 p.m.): Feeding. (7th August, 1915): Firm, hot swelling about the size of the hand had formed at the seat of inoculation. (8th August, 1915): M.T. 102.4°, E.T. 101°, pulse 64, respiration 12. (9th August, 1915): M.T. 101°, E.T. 101.4°, pulse 60, respiration 14. Animal fallen off in condition; tucked up; gait stiff; secondary swelling in sternal region, extending on to the forearms; this swelling pits on pressure; consistence doughy; mucous membrane paler than usual; hind limbs also swollen. (11th August, 1915): Swelling going; temperature, pulse, and respiration normal. (12th August, 1915): Swelling at seat of inoculation fluctuating; swelling on hind limbs almost gone. (13th August, 1915): Abscess at seat of injection has burst, with a very disagreeable yellowish discharge from a small wound; still slight swelling present on forearms. (18th August, 1915): An exacerbation of temperature 102.8°, probably accidental. (19th August, 1915): Still discharge present from the wound; mucous membrane slightly pale. (28th August, 1915): Only small wound present at seat of inoculation. Horse temperatured daily and kept under observation until 7th October, 1915. No further clinical symptoms were recorded until it was discharged from experiment.

(9th August, 1915): *Horse* 8752, chestnut gelding, condition poor. (2.46 p.m.): Injected subcutaneously with a watery extract of 100 *Gastrophilus* larvae (weight 31 grm.) (horse 2410, 6th August, 1915).

(2.47 p.m.): Shaking head several times; smelling the ground; chewing movements. (2.50 p.m.): Yawning; chewing movements; looking at near-side. (2.56 p.m.): Yawning and shaking head and body. (2.58 p.m.): Spreading the legs out and passing a fair quantity of a clear, brownish urine. (3.0 p.m.): Rubbing nose on front limbs; blowing the nose several times; chewing movements; repeatedly biting at sides. (3.20 p.m.): Drops of a watery discharge from eyes and nostrils; shaking head; masticatory movements. (3.30 p.m.): Clear watery discharge, yellowish in colour, dripping from the nose; this is mixed with some foam and mucous material; chewing movements. (3.35 p.m.): Defaecating. (3.45 p.m.): Watery discharge still copious from nose; respiration not increased. (4.5 p.m.): Respiration increased in frequency and abdominal.

Animal placed in a loose-box, making attempts at feeding. (4.30 p.m.): Lying in sternal position; rises on being approached; increased respiration still present; pulse weak and nearly imperceptible; temperature 101.2°. (8.0 p.m.): Animal standing quite quiet, but somewhat dull, and not feeding well. (10th August, 1915): Animal still somewhat dull and not feeding too well; a very painful, hot swelling at the seat of inoculation; a secondary oedematous swelling—which pits on pressure—is present in the lower third of the neck and extends on to the breast. Morning temperature 101.6°; evening temperature 101.4°; respiration 14; pulse 51. Animal goes lame in near front. (11th August, 1915): Swelling now extends on to forearms; gait somewhat uncertain; morning temperature 102°; evening temperature 101.4°; pulse 51; respiration 14. (12th August, 1915): Conjunctiva pale; swelling at seat of inoculation, fluctuating. It was opened and found to contain a very offensive, dirty material, mixed with some blood. Animal looks thin. Temperature normal; pulse 51; respiration 18. (13th August, 1915): A very offensive discharge escaping from the abscess cavity. (19th August, 1915): Swelling in sternum and legs has disappeared; lameness gone. Conjunctival mucous membrane somewhat pale. Horse temperatured daily and kept under observation until 23rd September, 1915. No further clinical observations were made.

Conclusions.—The experiment was not carried on further, because it was found in a later experiment dealing with the toxicity of the individual species of larvae that even 20 *Gastrophilus* larvae (injected into horse 9595 and horse 9763) may kill horses, whereas, when the same experiment was repeated in horse 9646 and horse 10015 only slight symptoms of an intoxication were shown. In experiment No. 2, horse 8971, injected with 20 *Gastrophilus* larvae, showed far more acute symptoms than horse 8752, inoculated with 100 *Gastrophilus* larvae subcutaneously, whereas horse 9556 and horse 10364 (see later), also inoculated with the watery extract from 20 *Gastrophilus* larvae subcutaneously, showed no symptoms of an intoxication. The abscesses which formed at the seat of inoculation had a very offensive but somewhat characteristic odour.

(c) *Drenching.*—(13th August, 1915): Horse 8806, bay gelding, condition moderately poor. (2.45 p.m.): Drenched with the watery extract of 500 *Gastrophilus* larvae and 500 c.c. physiological water (70 larvae—horse 9407, 10th August, 1915. 130 larvae—horse 9403, 11th August, 1915. 200 larvae—horse 9402, 12th August, 1915. 100 larvae—horse 9410, 6th August, 1915).

(2.48 p.m.): Chewing movements; yawning and rubbing nose against front legs. (2.58 p.m.): Chewing movements; stands quite quiet. (3.30 p.m.): Animal showed nothing further and was sent back to the stable. Horse was temperatured daily and kept under observation until 23rd September, 1915. No symptoms were recorded against it until discharge from experiment.

(23rd September, 1915): Horse 8873, chestnut mare, condition poor. (3.15 p.m.): Drenched with the watery extract of 1000 *Gastrophilus* larvae and 1000 c.c. water. (780 larvae—horse 9338, 23rd September, 1915; 220 larvae—horse 9504, 20th September, 1915).

(3.20 p.m.): Small quantity of normal faeces passed. (3.25 p.m.): Drops of perspiration noticed on nose, sides of abdomen, in the region of the perineum, and inside of the quarters. (3.30 p.m.): Defaecates and grunts; swishing its tail; restless; moving from side to side and continually lifting and changing the position of the limbs; walking in a circle; inside of the thighs wet with perspiration, also the roots of the ears, face, and quarters. (3.35 p.m.): Walking round and attempting to lie down; breathing laboured and increased in frequency; anxious expression on the face; drops of perspiration still trickling from the body; small quantity of faeces passed. (3.47 p.m.): Animal goes down, lying stretched out, attempting to roll; rises and is then placed in a loose-box. (3.50 p.m.): Found lying in sternal position and immediately after was found lying stretched out; this was repeated several times; animal wet with perspiration and still restless. (4.30 p.m.): Standing, but somewhat restless and exhausted;

respiration still increased and laboured. (6.15 p.m.): Lying in sternal position; does not rise on being approached; dull, but quite quiet. (8.0 p.m.): Animal still somewhat dull and not feeding. (24th September, 1915): Animal seems to have recovered completely and feeding. This horse was temperatured daily and kept under observation until 2nd November, 1915. No other symptoms were recorded until it was discharged from experiment.

Conclusions.—(1) It was found that horse 8806, drenched with watery extract of 500 *Gastrophilus* larvae, showed very slight symptoms of an intoxication, which lasted about 15 minutes. (2) It was found that horse 8873, drenched with watery extract of 1000 *Gastrophilus* larvae, showed very acute symptoms of an intoxication, which lasted about four hours.

Experiment No. 3.—To ascertain whether intoxication by *Gastrophilus* larvae occurs when they are injected into (a) mule, (b) donkey, (c) cattle, (d) sheep, (e) goat, (f) dogs, (g) rabbits, (h) guinea-pigs.

(a) To ascertain whether the watery extract of *Gastrophilus* larvae could produce symptoms of an intoxication when injected into the mule. (29th June, 1917): Mule 11375, brown mare, very old, condition poor. (10.40 a.m.): Injected intrajugularly with the watery extract of one *Gastrophilus* larva in 20 c.c. of physiological water.

(10.45 a.m.): Quivering of the skin over the whole body and of the tail noticed; passes a quantity of normal faeces; animal somewhat restless.

(10.50 a.m.): Licking movements; shifting the hind limbs frequently; beads of perspiration running from root of ears, sternal region, perineum, and inside of thighs; lifts the tail, and passes a quantity of faeces, somewhat softer in consistency. (10.52 a.m.): Licking movements; perspiration more profuse; hanging its head and appears somewhat excited; grinding the teeth and looking round at the sides; tremor still present. (10.55 a.m.): Shifting legs frequently, and again passing a quantity of soft faeces. Temperature 100.2°; pulse accelerated and softer; respiration slightly increased and abdominal. (11.0 a.m.): Continually swishing the tail; licking movements; again passes a quantity of soft faeces. (11.10 a.m.): Tail slightly raised; ears drawn backwards; head hanging; animal appears dull; perspiration less profuse; passes a quantity of watery faeces of foetid odour and dirty yellowish in colour. Observed to yawn frequently. (11.15 a.m.): Animal stands fairly quiet; no perspiration noticed; brought back to loose-box; does not feed. (12.0 p.m.): Animal seems to have recovered; not feeding well. (1st July, 1917): Animal feeds fairly well. (3rd July, 1917): At the site of inoculation (off jugular vein) a very firm thrombus about the thickness of a man's wrist can be felt, extending down into the chest.

(10.35 a.m.): Mule again injected intrajugularly with the watery extract of 5 *Gastrophilus* larvae in 20 c.c. physiological water.

(10.40 a.m.): Noticed to yawn frequently; licking movements. (10.42 a.m.): Lifts tail and passes a quantity of soft faeces. (10.44 a.m.): Swishing tail frequently; passes a quantity of watery faeces. (10.45 a.m.): Ditto. (10.46 a.m.): Ditto. Shaking the body and head; looking round at and biting its sides; increased abdominal respiration and watery discharge from nose and eyes; pulse increased in frequency and soft. (10.47 a.m.): A great quantity of watery faeces passed, foetid and of a dirty yellowish colour; mule restless, frequently changing the position of its limbs. (10.49 a.m.): Ditto. (10.52 a.m.): Ditto. (11.0 a.m.): Mule again passes a great quantity of watery faeces. (11.5 a.m.): Respiration more laboured; shaking and rubbing the head against the post frequently; stands fairly quiet. (11.15 a.m.): Animal placed in a loose-box; does not feed; appears distinctly dull and stands with the head hanging. (12.0 p.m.): Mule down, lying in the sternal position, with eyes half closed; rises immediately when threatened. (2.0 p.m.): Again found lying in the sternal position. (4.0 p.m.): Mule found standing, but does not feed.

Conclusions.—(1) Symptoms of an intoxication, following the injection of watery extracts of *Gastrophilus* larvae, occurred in the mule, and were identical to those observed in the horse. (2) One

larva and five larvae injected intrajugularly did not kill, as was the case in the horse, but produced acute symptoms, the most notable being that of diarrhoea.

(b) To ascertain whether the watery extract of *Gastrophilus* larvae from horses could produce symptoms of an intoxication when injected into donkeys. (5th September, 1917): *Donkey* 8479, injected intrajugularly with the watery extract of 5 *Gastrophilus* larvae (horse 11032) in 20 c.c. physiological water.

(3.15 p.m.): Injected. (3.20 p.m.): Lifts its tail and passes a quantity of normal faeces. (3.45 p.m.): Animal distinctly restless; moves from side to side; observed to kick at the abdomen; dull; ears drawn backwards; masticatory movements; anxious expression on face; swishing the tail; slightly increased abdominal respiration. (4.0 p.m.): Slight lachrymation; blowing the nose rather frequently. (4.30 p.m.): Feeding slightly; still increased respiration; dull; ears still drawn backwards. (8.0 p.m.): Donkey feeds, but appears somewhat dull. (7th September, 1917): A firm, hot and painful swelling has formed at the seat of inoculation (septic thrombo-phlebitis); otherwise appears normal.

(6th September, 1917): *Donkey* 9810, injected intrajugularly with the watery extract of 25 *Gastrophilus* larvae (horse 11032) in 25 c.c. physiological water.

(2.30 p.m.): Injected. (2.35 p.m.): Somewhat restless; yawns; twitches the lips. (2.40 p.m.): Profuse perspiration in the form of beads from ears, nose, and inside of thighs; lachrymation; ears drawn backwards; anxious expression on face; respiration increased. (2.45 p.m.): Respiration further increased and laboured; sways from side to side; head hangs; dull and listless. (2.55 p.m.): Animal sways from side to side as if wanting to fall; perspiration further increased; eyes half closed; pulse accelerated and small; lachrymation further increased; serous discharge from both nostrils fairly copious. (3.0 p.m.): Animal sways when it walks forwards; chewing movements; rubs its nose against the front limb. (3.30 p.m.): Dull; head hanging; respiration more laboured; region of axilla, thighs, and round about the nose quite wet from profuse perspiration; animal walks about listlessly and aimlessly in the box, staggering from side to side. (5.0 p.m.): Not recovered completely and somewhat dull. (7th September, 1917): Completely recovered. (10th September, 1917): A painful hot swelling formed at the seat of injection (septic thrombo-phlebitis).

Conclusions.—(1) Symptoms of an intoxication, following on the injection of watery extract of *Gastrophilus* larvae, were identical (only less acute) to those observed in the horse. (2) The donkey inoculated intrajugularly with 5 *Gastrophilus* larvae showed slight symptoms, whereas the donkey injected with 25 *Gastrophilus* larvae showed more acute symptoms of an intoxication. (3) Donkeys appear to be much less susceptible than horses, and less susceptible than the mule, to the intrajugular injection of the watery extract.

(c) *Cattle.*—(3rd August, 1915): *Cattle* 3371, red heifer, condition fair. (12.27 p.m.): Injected intrajugularly with the watery extract of one *Gastrophilus* larva (weight 500 mg.) in 20 c.c. physiological water.

(12.28 p.m.): Passes small quantity of urine and faeces of normal colour and consistence. (1.0 p.m.): Animal showed no symptoms. (6th October, 1915): Discharged from experiment. No symptoms were observed during this period.

(13th September, 1916): *Cattle* 3196, red ox, condition rather poor. (2.30 p.m.): Injected intrajugularly with the watery extract of 10 *Gastrophilus* (*equi*) larvae in 20 c.c. physiological water.

(2.50 p.m.): Slightly increased respiration, probably due to the animal's struggling to get loose. (29th September, 1916): Discharged from experiment. No symptoms were observed during this period.

(5th July, 1917): *Cattle* 3628, black and white bull. 2 years, condition rather poor.

(10.50 a.m.): Injected intrajugularly with 30 *Gastrophilus* larvae and 20 c.c. physiological water. No symptoms were observed. (18th July, 1917): Discharged from experiment.

Conclusions.—(1) Three cattle injected intrajugularly with the watery extracts of 1, 10, and 30 *Gastrophilus* larvae respectively showed no symptoms of an intoxication.

(d) *Sheep*.—(13th August, 1915: *Sheep* 7362, wether, condition fair. (2.32 p.m.): Injected intrajugularly with watery extract of one *Gastrophilus* larva in 20 c.c. physiological water.

(2.35 p.m.): Blowing; standing with head lowered; increased respiration, becoming more marked; swaying to side and nearly falling; defaecating. (2.36 p.m.): Going back and staggering. (2.37 p.m.): Finally going down; lying in sternal position. (2.40 p.m.): Going on to left side and lying with front legs slightly drawn to and under the body; respiration increased and abdominal. Salivation; slight mucous discharge from nose; eyes half closed. (2.45 p.m.): Respiration further increased; movement of nostrils observed with inspiration. (2.47 p.m.): Up again in sternal position, with head turned to right side of body. (2.50 p.m.): Respiration further increased; more discharge from nostrils; head turned to right side of body; mouth open, and tongue hanging out; when picked up and placed on legs, animal staggers, able to stand for short while only, and then goes down again. (2.55 p.m.): Up again and staggering; breathing with mouth open and observed to cough slightly twice; tongue hanging out; marked abdominal respiration; staggering about; restless; anxious expression on face. (2.56 p.m.): Down again, lying in sternal position, and breathing with mouth open. (2.58 p.m.): Up again; staggering aimlessly about; mouth open; staggering in half circles, then forwards. (3.0 p.m.): Down again, sternal decubitus; breathing with mouth open; mouth full of foamy saliva; metallic sounds heard with inspiration and expiration; mucous discharge hanging from both nostrils. (3.5 p.m.): Slight cough; up again; standing quiet, until falling over on to side; watery discharge from both eyes. (3.11 p.m.): Up again and then down; slight coughing sounds heard; bronchial respiration becomes more marked. (3.12 p.m.): Up and down several times. (3.30 p.m.): Breathing not so laboured; mouth closed; still staggering about aimlessly, but not so weak. (3.40 p.m.): Much stronger; respiration less laboured and slowed down. Still slight staggering gait. (4.10 p.m.): Respiration still increased, but not so laboured; improvement in gait; sent to box. (4.30 p.m.): Standing quiet; respiration still increased; not feeding. (14th August, 1915): Seems to have recovered. (6th October, 1915): Discharged from experiment.

(13th September, 1916): *Sheep* 10220, condition fair. (2.32 p.m.): Injected intrajugularly with the watery extract of 5 *Gastrophilus* (equi) larvae in 20 c.c. physiological water. The extract amounted to 17 c.c.

(2.35 p.m.): Shaking head; abdominal respiration. (2.38 p.m.): Blowing and straining slightly; inspiratory dyspnoea, with mouth open and nostrils dilated. (2.40 p.m.): Swaying slightly; licking movements; mouth opened with each inspiratory movement. (2.43 p.m.): Swaying slightly and hanging head. (2.45 p.m.): Passes a quantity of normal faeces. (2.46 p.m.): Sheep shows some licking movements; breathing with mouth open and respiration further increased; moving head a few times from side to side and slightly downwards. (2.50 p.m.): Urine dribbling and serous fluid dribbling from both nostrils. (2.55 p.m.): Walking about in a slightly dazed manner. (3.0 p.m.): Respiration further increased, but not so laboured; animal standing. (3.15 p.m.): Animal still breathing with mouth open; ears hanging; walks about fairly well; takes no notice of surroundings; animal appears slightly tympanitic; (3.25 p.m.): Respiration still increased and laboured; more tympanitic on left side. (4.30 p.m.): Still showing inspiratory dyspnoea; animal up to now has not gone down. (4th September, 1916): Looks dull and not feeding well. 15th September, 1916): Seems to have recovered. (25th September, 1916): Discharged from experiment.

(9th May, 1917): *Sheep* (no number), Merino, wether, full-mouth, condition fair, temperature 104°. (3.0 p.m.): Injected intrajugularly with 18 c.c. watery extract of 10 *Gastrophilus* larvae (weight 4 grm.) in 20 c.c. physiological water.

(3.8 p.m.): Shows licking movements and shakes the head violently. (3.10 p.m.): Shakes the head, which is lowered and slightly turned to the one side; respiration is increased in frequency. (3.12 p.m.): Repeated licking movements; respiration further increased in frequency and more laboured; nostrils moving. (3.15 p.m.): Standing in a listless fashion; does not move when one approaches it; respiration more laboured; every now and then bronchial noises heard. (3.17 p.m.): Good quantity of mucous material escaping from the nostrils; slight salivation present. (3.25 p.m.): Mucous discharge from nostrils now fairly copious; passes a fair quantity of normal faeces; gait shows nothing unusual. (3.30 p.m.): Observed to open the mouth with every inspiratory movement, and protrudes the tongue; head still hanging low. Temperature 105.2°; passes a quantity of normal faeces. (3.35 p.m.): At times still breathes with its mouth open; a foamy saliva present on the edges of the lips; mucous discharge from nostrils still copious. (4.0 p.m.): Animal distinctly dull; respiration accelerated, but less laboured; does not feed. (5.0 p.m.): Ditto. (8.0 p.m.): Respiration normal; not feeding. (10th May, 1917): Animal feeds and seems to have recovered completely.

(5th July, 1917): *Sheep* 10764, Merino, wether, full-mouth, condition fair. (10.53 a.m.): Injected intrajugularly with the watery extract of 30 *Gastrophilus* larvae in 20 c.c. physiological water.

(10.55 a.m.): Head hanging low; abdominal respiration. (10.58 a.m.): Respiration accelerated and laboured. (11.0 a.m.): Passes a quantity of faeces of normal colour and consistence; a watery discharge from nostrils; goes down and lies in sternal position, with one front leg extended and head turned to its left side, mouth resting on the ground. Inspiratory and expiratory dyspnoea, with grunting noises every now and then. (11.5 a.m.): Sheep still occupying same position and breathing with mouth open. (11.10 a.m.): Head and body quite limp; respiration very slightly visible; eye reflex still slightly present; animal dies almost immediately without showing further symptoms.

Conclusions.—(1) Four sheep injected intrajugularly with the watery extracts of 1, 5, 10, and 30 *Gastrophilus* larvae, respectively, showed acute symptoms of intoxication, resulting in the death of the one (receiving 30 larvae) in 17 minutes, and the recovery of the others in about two hours. (2) The number of larvae injected does not seem to influence the severity of the symptoms. Sheep injected with one larva showed more acute symptoms than sheep injected with ten larvae. (3) Symptoms commenced, within a few minutes, with licking movements, followed by inspiratory and expiratory dyspnoea, accompanied by salivation and watery discharge from the nostrils, together with muscular weakness. A period of excitement is followed by a period of depression.

(e) *Goats.*—(13th August, 1915): *Goat* 5031, condition fair. (2.15 p.m.): Injected intrajugularly with the watery extract of one *Gastrophilus* larva (weight 400 mg.) in 20 c.c. physiological water.

(2.20 p.m.): Passes small quantity of faeces. (2.22 p.m.): Respiration slightly increased; bleats once. (2.26 p.m.): Licking and chewing movements. (3.0 p.m.): Seems to have recovered.

(13th September, 1916): *Goat* 5292, condition fair. (2.35 p.m.): Injected intrajugularly with 5 *Gastrophilus* (*equi*) larvae in 20 c.c. physiological water. The extract amounted to 19 c.c.

(2.38 p.m.): Respiration markedly increased, but superficial. (2.40 p.m.): Shaking body and bleating. (2.42 p.m.): Shaking and lifting head; animal remains standing quiet, even when one approaches it and threatens it.

(2.45 p.m.): Stretches hind limbs backwards, lifting head upwards, and pulled slightly backwards, and bleats several times. (2.48 p.m.): Shaking head; pitiful bleating which is not loud; tongue slightly protruding. (2.52 p.m.): Bleats and stretches hind limbs backwards; respiration still markedly increased, but superficial. (2.55 p.m.): Standing with head in corner and slightly lowered; assumes sternal position. (3.5 p.m.): Still lying in sternal position; blowing; slight licking movements. (3.25 p.m.): Standing; shows slight improvement in respiration. (4.0 p.m.): Standing with head slightly lowered; listless and does not take much notice of surroundings. (14th September, 1916): Animal looks somewhat dull and not feeding too well, has been observed to ruminate. (25th September, 1916): Discharged from experiment.

(9th May, 1917): Goat 4813, condition fair, full-mouth; temperature 104.6°. (3.0 p.m.): Injected intrajugularly with 17 c.c. watery extract of 10 *Gastrophilus* larvae (weight 4 grm.) in 20 c.c. physiological water.

(3.5 p.m.): Observed to shake the head violently several times. (3.7 p.m.): Again repeated. (3.8 p.m.): Blowing the nose and shaking the head frequently. (3.9 p.m.): Slight abdominal respiration. (3.13 p.m.): Respiration further increased in frequency and difficulty. (3.15 p.m.): Bronchial noises heard with inspiratory movement; eyes half closed; standing quite quiet; appears dull and does not move away when approached. (3.18 p.m.): Shows some weakness in hindquarters and staggers slightly from side to side. (3.20 p.m.): Eyes still half closed and dull; a very distinct jugular pulse observed. (3.30 p.m.): Temperature 104.6°. (3.35 p.m.): Animal more attentive; respiration still more frequent than normal. (4.0 p.m.): Shows further improvement. (8.0 p.m.): Seems to have recovered completely.

Conclusions.—(1) Goats injected intrajugularly with extract of one *Gastrophilus* larva showed slight symptoms of an intoxication, which passed off in about half an hour. (2) Goats injected intrajugularly with extract of five and ten *Gastrophilus* larvae showed symptoms of an intoxication, commencing a few minutes after injection and lasting about two hours. (3) Symptoms were of a similar nature as those observed in sheep, but were not so acute.

(f) *Dogs.*—(3rd July, 1917): Dog 1316, red and white half-bred pointer, condition fair. (10.30 a.m.): Injected intrajugularly with the watery extract of five *Gastrophilus* larvae in 20 c.c. physiological water. Dog showed no symptoms of an intoxication and was discharged on 18th July, 1917.

(5th July, 1917): Dog 1326, half-bred Irish terrier, condition fair. (10.55 a.m.): Injected intrajugularly with the watery extract of 15 *Gastrophilus* larvae in 20 c.c. physiological water.

(10.58 a.m.): Commences to strain and vomits once, bringing up a fair quantity of a custard-like material. (11.0 a.m.): Ditto. (11.15 a.m.): Dog showed no other symptoms, and was discharged on 18th July, 1917.

(4th October, 1917): Dog 1320: Injected intrajugularly with the watery extract of 20 *Gastrophilus* larvae in 20 c.c. physiological water. No symptoms were observed. (14th October, 1917): Dog was discharged from experiment.

Conclusions.—(1) Of three dogs injected intrajugularly with the watery extract of 5, 10, and 20 *Gastrophilus* larvae, respectively, the first showed no symptoms; the second vomited twice in the space of two minutes, but showed no other symptoms; the third dog showed no symptoms.

(g) *Rabbits.*—To ascertain whether the watery extracts of *Gastrophilus* larvae show any symptoms of intoxication when injected (1) intravenously. (2) subcutaneously. (3) intraperitoneally.

(1) *Intravenously*—

Date of Experiment.	No. of Rabbit.	Dose.	Result.
22.9.16	1 6	3 c.c. of 24 G. larvae —48 phys. water	5 died after 18 hours; 1 died after 21 hours.
7.3.17	7 18	2 c.c. of 24 G. larvae —48 phys. water	1 died after 17 hours; 2 after 18 hours; 2 after 19 hours; 1 after 21 hours; 2 after 24 hours; 3 after 42 hours; 1 developed an abscess at the seat of inoculation.
8.3.17	19 26	1 c.c. of 24 G. larvae —48 phys. water	1 died after 21 hours; 1 after 23 hours; 1 after 5 days; 1 after 7 days; 4 were reinoculated later.

In the above experiment none of the rabbits showed any symptoms of an intoxication, following on the injection, as were observed in the other animals. They commenced to die from the effects of the injection after the seventeenth hour. Death in no case was preceded by any characteristic symptoms. Shortly before death rabbits appeared dull and did not feed. Respiration was quickened and animal became comatose and died. One larva pounded up with 2 c.c. physiological water usually yields about 3 c.c. of filtrate when passed through muslin. When the contents of one larva, i.e. 3 c.c., is injected (e.g. experiment on 22nd September, 1916) mortality was 100 per cent. When 2 c.c. is injected (e.g. experiment on 7th March, 1917) similar results were obtained; one rabbit in this experiment, however, did not die, but developed a large abscess at the seat of inoculation. The dose must have been injected subcutaneously instead of intravenously. When 1 c.c. is injected (e.g. on 8th March, 1917) the percentage of mortality was only 50.

Post-mortem lesions.—The abdominal cavity contains a varying quantity of a blood-stained fluid, and in the pericardial and pleural cavities in the majority of cases a similar fluid was present; also hyperaemia of kidneys and spleen. In some there was hyperaemia of the stomach and lungs. The peritoneal lining had a pink flush.

Conclusions.—(1) Watery extracts of *Gastrophilus* larvae when injected intrajugularly in sufficiently large doses proved to be very fatal to rabbits. (2) There were no symptoms of an intoxication such as those observed in horses directly after the injection. (3) Death only took place, at the earliest, after the seventeenth hour, and occurred up to the fifth and seventh days. (4) The course of the disease and post-mortem lesions point to a bacterial infection introduced with the non-sterile watery extract of *Gastrophilus* larvae.

(V.B.—Further experiments were undertaken to elucidate this point.)

(2) Subcutaneously—

Date.	No. of Rabbits.	Dose.	Result.
13.9.16	1, 2	5 G. larvae in 5 c.c. phys. water	Large abscesses at seat of inoculation.
22.9.16	3-8	3 c.c. of 24 G. larvae in 48 phys. water	" "
8. 3.17	9-11	1 c.c. of 24 G. larvae in 48 c.c. phys. water	" "

Conclusions.—(1) No symptoms of an intoxication were observed directly after subcutaneous inoculation. (2) Abscesses formed at varying periods after the injection, due to the inoculation of a non-sterile watery extract.

(3) Intraperitoneally—

Date.	No. of Rabbits.	Dose.	Result.
1.9.16	1, 2	3 c.c. of 1 G. larva in 5 c.c. phys. water	One died after 26 hours; one died after 72 hours.
22.9.16	3 8	3 c.c. of 24 G. larvae in 48 c.c. phys. water	One died after 48 hours; five re-injected on 16.10.16.

Conclusions.—(1) No symptoms of an intoxication were observed directly after intraperitoneal inoculation. (2) Of eight rabbits injected, three died after 26, 48, and 72 hours, respectively, showing similar symptoms and post-mortem lesions to those described in the case of intravenous injection. Death was probably due to a bacterial infection; the mortality in the case of intraperitoneal injection was much less than in the case of intravenous injection.

(h) Guinea-pigs.—(1) Subcutaneously—

Date.	No. of Guinea-pigs.	Dose.	Result.
1.9.16	1, 2	1 G. larva in 5 c.c. phys. water	27.9.16, killed on account of large abscesses.
22.9.16	3, 4	3 c.c. of 4 G. larvae in 8 c.c. phys. water	Both killed on account of large abscesses.
25.9.16	5, 6	5 G. larvae in 10 c.c. phys. water	" "

(2) *Intraperitoneally*—

Date.	No. of Guinea-pigs.	Dose.	Result.
13.9.16	1, 2	1 G. larva in 5 c.c phys. water	Died of peritonitis.
22.9.16	3, 4	3 c.c. of 4 G. larvae in 8 c.c. phys. water	„ „

(3) *Intravenously*—

Date.	No. of Guinea-pigs.	Dose.	Result.
25.4.17	1, 2	1 c.c. of 1 G. larva in 10 c.c. phys. water	None.

Conclusions.—(1) In the twelve guinea-pigs injected in different ways, no symptoms of an intoxication were observed immediately after the injection. (2) In the cases of subcutaneous injection the guinea-pigs thrived well, but developed large abscesses at the seat of inoculation. (3) in the cases of intraperitoneal injection the guinea-pigs died of peritonitis. *N.B.*—(4) Guinea-pigs were injected subcutaneously and intraperitoneally with about five times the dose which killed horses intravenously without showing any symptoms of an intoxication.

Experiment No. 5 was undertaken to prove: (a) That the high percentage of mortality of the rabbits injected intravenously with the *watery extract* of *Gastrophilus* larvae is due to a bacterial infection, and that no such mortality occurs when the *filtrate* of the watery extract (through a Berkefeld filter) is used. (b) That such a filtrate through a Berkefeld still retains the toxin of the *Gastrophilus* larvae.

Rabbits were injected intravenously with *filtrate*, with the following controls:—(a) The same number of rabbits injected with the *extract* from which the filtrate was prepared. (b) Twelve guinea-pigs injected with the *filtrate* subcutaneously. (c) One horse injected with the *filtrate*.

Date.	No. of Animals.	Dose.	Result.
19.7.17	Rabbits 1, 2, 3	3 c.c. <i>filtrate</i> of 100 G. larvae and 2,000 c.c. water	No symptoms.
19.7.17 <i>Controls</i>	Rabbits 4, 5, 6	3 c.c. <i>extract</i> of 100 G. larvae and 2,000 c.c. water	All three rabbits died after the nineteenth hour.
19.7.17 <i>Controls</i>	H.11334	50 c.c. <i>filtrate</i> of 100 G. larvae and 2,000 c.c. water	Acute symptoms of intoxication ; dead within 1½ hours.
19.7.17 <i>Controls</i>	12 Guinea- Pigs	3 c.c. <i>filtrate</i> of 100 G. larvae and 2,000 c.c. water in- jected subcutaneously	No symptoms.

Experiment repeated on :—

27.7.17	Rabbits 7, 8, 9	3 c.c. <i>filtrate</i> of 150 G. larvae and 3,000 c.c. water	No symptoms.
27.7.17 <i>Controls</i>	Rabbits 10, 11, 12	3 c.c. <i>extract</i> of 150 G. larvae 3,000 c.c. water	One died after 2 days ; one died after 4 days ; one developed abscess locally.

In each case cultures were made on agar, both from the filtrate and extract. In both cases the "*filtrate*" culture was sterile after forty-eight hours' incubation at 37° C., whereas the "*extract*" cultures showed well-defined circular greyish white colonies, about the size of a millet seed. Stained preparations of these showed a pure coccus infection. From the dead rabbits, smears were made from the blood and spleen, and in those rabbits which died early numerous small cocci were seen, sometimes occurring in chains of two, three, and even six. In those rabbits, which died after two days only, a few cocci were detected in the smears from blood and spleen.

(b) That such a *filtrate*, through a Berkefeld filter, still retains the toxin, was shown in the case of *control* horse 11334, which commenced to show acute symptoms of an intoxication a few minutes after the injection, and died within one and a half hours.

(2.40 p.m.): *Horse* 11334 was injected. (2.50 p.m.): Animal commences to strain; only passes a few balls of faeces; tenesmus continues; animal distinctly restless; moving from side to side. (3.0 p.m.): Shows inspiratory and expiratory dyspnoea; passes a quantity of watery faeces; beads of perspiration trickling from the body; head hanging and anxious expression on the face. (3.15 p.m.): Horse shows marked expiratory dyspnoea; lower lip quite limp; tongue hanging out of mouth; profuse perspiration; animal sways from side to side; pulse imperceptible; profuse salivation; passes a good quantity of watery faeces. (3.30 p.m.): Animal goes down and lies spread out; very weak, with eyes half closed; kicks out with legs; strains; unable to regain sternal position. (3.45 p.m.): Animal still lying spread out; shows marked and laboured abdominal respiration; eyes half closed. (3.55 p.m.): Respiration jerky and less laboured; animal strains every now and then; gasps and dies a few seconds afterwards.

That such a filtrate does contain the *oestrin toxin* is further borne out by the following cases in some of the above experiments. Here such filtrates were injected subcutaneously instead of intravenously.

(7th September, 1916): *Horse* 10400, 20 c.c. of 60 *Gastrophilus* larvae and 100 c.c. water. Symptoms of an intoxication commenced about fifteen minutes after injection, not very acute, and lasting about three hours; dyspnoea; diarrhœa; followed by depression and dullness; not feeding well; no swelling.

(7th November, 1916): *Horse* 10683, 100 c.c. 50 *Gastrophilus* larvae and 300 c.c. water. Ten minutes after injection commenced to show symptoms, viz., restlessness, diarrhœa, and dyspnoea, followed by depression; not feeding.

(13th July, 1917): *Horse* 11384, 250 c.c. 100 *Gastrophilus* larvae, in 1000 c.c. water. Five minutes after injection the animal began to show symptoms of an intoxication in the form of restlessness, dyspnoea, perspiration, diarrhœa, followed by depression; symptoms lasted about four hours and not acute; no swelling.

Discussion.—From the above experiments it will be clearly noted that the watery extracts from the *Gastrophilus* larvae contain toxins which produce very definite and specific symptoms either when given to the horse subcutaneously, intravenously, or per os. Similar symptoms, but less acute, were observed in the mule and donkey. In sheep and goats symptoms of acute intoxication were also seen, differing from those in the horse but slightly. In dogs, cattle, rabbits, and guinea-pigs no such symptoms were observed. The high percentage of mortality occurring in rabbits was due to a bacterial infection introduced with the extract.

On account of the great diversity of opinion existing about the intoxication caused by animal parasites, the symptoms observed have been carefully noted and given in full. These symptoms in the horse varied in the different animals injected. They seem to come on within a couple of minutes, and, in horses which did not succumb, they disappeared again in from two to six hours. They set in with a sense of nausea, yawning, shaking the head and body, licking the lips, irritation of the skin (rubbing the nose against the legs or on posts), followed by restlessness, moving from side to side, lifting and shifting the legs and position frequently, swishing the tail, and looking and biting at the sides; respiration increases in frequency and becomes more laboured, leading to dyspnoea; they are accompanied by tenesmus; animal becomes prostrated and anxious. At the same time there is profuse sweating and salivation, frequent micturition and defaecation, the faeces gradually becoming more and more watery in consistence; watery discharge from eyes and nose. The animal enters the stage of collapse; the body temperature is cold, but the temperature per rectum remains normal; the pulse, which is small and frequent, becomes imperceptible; the injected mucous membranes become somewhat cyanotic; the animal sways and staggers, goes down with convulsions, and dies almost immediately. In those cases which do not succumb the period of restlessness is followed by a period of depression and exhaustion. Improvement in respiration, pulse, etc., soon sets in and the animal appears to recover from the more acute symptoms in from two to four hours, but still remains dull, and does not feed, or only very slightly. In the subcutaneously injected horses very painful

swellings usually form within twenty-four hours. These eventually become abscesses, discharging a very disagreeable, offensive, but somewhat characteristic yellowish, granular material. This is sometimes mixed with some haemorrhage. In a few cases there was secondary swelling and slight fever present, which disappeared in about three days.

From the post-mortem lesions no definite conclusions could be drawn as to whether these toxins give rise to specific anatomical changes. No microscopical examination was made. Heart lesions were present in all cases that succumbed to an intoxication. In some there were ecchymoses of epicardium and both endocardiums; in others only slight ecchymoses of the left endocardium were noted. In all cases *Gastrophilus* larvae were present in the stomach. No other definite post-mortem lesions were present.

Of the equidae, the horse appears to be the most susceptible to the toxin. Of the ruminants, the sheep appears to be more susceptible than the goat. In rabbits and guinea-pigs no symptoms of an intoxication were observed; there was a high percentage of mortality in the former. This was shown to be due to bacterial infection, introduced with a non-sterile watery extract. It was also shown that the toxin passed through a Berkefeld filter. Symptoms of an intoxication by extracts from animal parasites are also described by other investigators (given above). Weinberg in his researches alludes to a variation in the degree of the toxicity of the substance liberated by the helminths: this, amongst other things, depends on the susceptibility of the host. Seyderhelm and Seyderhelm speak of a toxic action of the *Gastrophilus* larvae, and regard it as absolutely specific for the horse and mule. Carré and Vallée observed acute symptoms of an intoxication when watery extracts of the *Gastrophilus* larvae were injected intrajugularly. The diversity of opinion existent in regard to the occurrence of an intoxication produced in animals by the extract of animal parasites might lend itself to be explained by one of the following suggestions:—*(a)* Varying susceptibility of the different species of animals to the toxins of the parasite used; *(b)* method of preparation and treatment, concentration, dose, etc., of the extract employed; *(c)* the injection of a non-sterile watery extract, which may be followed by symptoms, and death after a certain incubation period. It might be mistaken for symptoms of an intoxication, as was the experience of Alessandrini, Paolucci, and Guerrini, who came to the conclusion that the ascarides fluids do not contain toxins (for small animals), but that death is due to the injection of such animals with fluids containing infectious germs. In the experiments above on rabbits the same conclusions were drawn, i.e. that a bacterial infection was responsible for the high percentage of mortality.

Hadwen and Bruce described more or less similar symptoms in cattle and sheep treated with the extracts of larval forms of *Hypoderma* and *Oestridae*. These authors, however, wish to explain the symptoms observed as acute and chronic forms of anaphylaxis, which they reproduced, and which coincide with Richet's definition. These reactions, according to them, can be induced by crushing and returning an extract of an animal's own larvae into the jugular, showing that the larvae living in the host made them more receptive. Natural cases of anaphylaxis are described, where no injections had been given and

where injury had ruptured the larvae subcutaneously, liberating their contents in sufficient quantities to produce shock. Hadwen and Bruce seem to be greatly influenced by the usual train of anaphylactic symptoms (e.g. peripheral irritation, dyspnoea, spasms and convulsions), and their observations are further supported by experiments made with small animals, which were sensitized with warble extracts, and showed signs of anaphylaxis following the second injection. The same assumptions could be made to explain the symptoms, etc., observed in horses, etc., as an anaphylaxis instead of a direct intoxication. Horses, mules, and donkeys would probably be more receptive on account of the presence of *Gastrophilus* larvae in the animal, and in the case of sheep by the presence of an allied genus (*Oestrus ovis*), which, however, is not very common in South Africa. But it is difficult to explain the symptoms observed in goats as anaphylaxis. How were these animals made more receptive previous to the injection of the watery extract of the *Gastrophilus* larvae? Slight, but somewhat indefinite symptoms of anaphylaxis were also observed in guinea-pigs, which were previously sensitized with watery extracts of *Gastrophilus* larvae. These experiments are still in progress. Anaphylactic phenomena have been successfully produced in animals with hydatid fluid by several authors since 1909. Most probably in all these cases anaphylaxis had been produced by a non-specific proteid present in the extract, corresponding with the volume of work on anaphylaxis carried out with antigens consisting of animal sera, egg-white and other proteins. The mere existence of toxic substances in the bodies of these parasites is no evidence that they excrete them, and it is not certain whether animal parasites do liberate fluctuating amounts of a specific antigen rendering the host hypersusceptible. Very little definite information has so far been obtained, and correspondingly much speculation and reasoning from analogies of serum anaphylaxis have been the bases of many inferences and theoretical suggestions. Muller interprets his work entirely in the sense of Friedberger's anaphylatoxin theory, in that he believes acute death, which follows immediately upon the *first* injection of bacteria and bacterial extracts (if the amount given be sufficient), to be due to the formation in the blood stream of a poison resulting from a reaction between plasma and bacteria, a process which is more rapid in the sensitized animals in whose blood stream antibodies are present in relatively higher concentration.

F. G. Novy and P. H. de Knuff in their paper "Anaphylatoxin and Anaphylaxis," December, 1916, with reference to the part dealing with the effects of intravenous injections of agar are of opinion that one general principle seems to underlie a host of so-called intoxications. Agar injected into guinea-pigs and rats may produce a typical anaphylactic shock and death, with the usual autopsy findings. The reaction which takes place was shown to be identical with that which occurred in a sensitized animal when shocked. The pathogenic or non-pathogenic organisms, dead or alive, as well as non-cellular substances (organic or inorganic) acting on serum in the test tube, or in the plasma in the living (host) body, create a disturbance, which finds its expression in anaphylatoxic poisoning. The in-vivo production of anaphylatoxin in agar-shocked rats and guinea-pigs was demonstrable by blood transfusion, and proof is thus given that the shock effects and death are due to this poison. In typical agar intoxications

the effects cannot be ascribed to agar itself, but to anaphylatoxin, which is made within the blood of the animal as a result of the disturbance set up by the presence of an alien substance.

Experiment No. 5.—To ascertain whether there is a difference in the toxic action produced by the *different species* of *Gastrophilus* larvae when the watery extracts of these are inoculated into horses. In 1914 Van K. R. Seyderhelm and R. Seyderhelm came to the conclusion that the toxic action of the *Gastrophilus haemorrhoidalis* is much more accentuated than that of *Gastrophilus equi* when injected into horses as extracts. In South Africa (see Mr. Bedford's paper) only three species have so far been identified, viz., *Gastrophilus equi*, *Gastrophilus pecorum*, *Gastrophilus nasalis*. *Gastrophilus haemorrhoidalis* has not yet been seen and described. However, experiments were undertaken to see whether any of the above species which occurred in South Africa was more toxic than the others.

Up to now, as will be seen in the foregoing experiments, it was found practically impossible to standardize the toxic dose for horses by injecting subcutaneously a certain number of *Gastrophilus* larvae. In some horses 20 *Gastrophilus* larvae showed very slight symptoms of an intoxication, in some more acute and in others fatal; whereas in one horse 100 *Gastrophilus* larvae only showed acute symptoms of an intoxication. For that reason it was decided in this experiment to inoculate the different species of larvae as follows:—To select only full-grown larvae and to inoculate an equal weight of larvae of each species.

(7th September, 1916): *Horse* 9595, bay gelding, condition very poor. (2.45 p.m.): Injected subcutaneously with the watery extract of 6.2 gm. of *Gastrophilus nasalis* larvae (horse 9611, horse 10434) in 20 c.c. physiological water. The watery extract amounted to 17 c.c.

(2.47 p.m.): Animal commences to kick rather violently with its hindlegs at the abdomen; moves restlessly about from side to side and stamps with the front limbs; grinding the teeth and biting at the sides. (2.50 p.m.): Swishing the tail and again kicking violently at the belly; grinding the teeth; ears drawn back. (2.55 p.m.): Blowing the nose; chewing movements; yawns several times. (2.58 p.m.): Restless; rubbing the side of the nose against the post or inner side of forelegs and kicking at abdomen. (3.0 p.m.): Straining; increased and laboured respiration; animal stands quiet, but sweats profusely, with drops running off the sides of the body, more marked on inner side of thighs and forearms; head hanging and eyes half closed. (3.2 p.m.): Tail raised; straining; swaying and staggering from side to side as if wanting to drop down; extremities cold; body quite wet from perspiration; pulse imperceptible; quivering of skin in places and spasms of the shoulder and quarter "group" of muscles. (3.5 p.m.): A foamy, stringy saliva from its mouth; also white flakes of foam mixed with mucus from the nostrils; head hanging still lower; marked abdominal respiration; small quantity of normal faeces, followed by some watery faeces, were passed; strains and grunts. (3.13 p.m.): Spasms of muscles more marked; animal staggers and has to be held up. (3.15 p.m.): Animal suddenly throws itself on its side and dies with symptoms of convulsions in a few minutes.

Post-mortem lesions.—Petechiae of larynx; marked ecchymoses and extravasations of left endocardium. Hyperaemia of lungs; hyperaemia of fundus of stomach; *Gastrophilus* larvae present.

(7th September, 1916): *Horse* 9763, bay gelding, condition very poor. (2.45 p.m.): Injected subcutaneously with watery extract of 6.2 gm. of *Gastrophilus equi* larvae (horse 9611, horse 10434) in 20 c.c. physiological water. The watery extract amounted to 19 c.c.

(2.50 p.m.): Grinding the teeth; chewing movements. (2.55 p.m.): Passed a couple of ounces of faeces, normal in colour and consistence; licking movements

of the lips; lifts the tail; lifts hind limbs alternately. (3.0 p.m.): Tail still raised; increased abdominal respiration, which becomes laboured; head hanging lower; eyes half closed; straining; sweating profusely; beads of perspiration trickling from its body, more marked on the inside of its thighs, forearms, and nose. (3.5 p.m.): Standing with a somewhat wry neck, which is held stiff; anxious expression on the face; presses forwards, standing with hind limbs apart; sways and staggers and has to be supported; foamy sero-mucous discharge from the nose. (3.10 p.m.): Straining and emits a grunt every now and then; respiration more laboured; very restless; staggers from side to side and presses forwards. (3.15 p.m.): Animal throws itself down, lies stretched out; yawns several times; pulls neck and head backwards, mouth open, and tongue hanging out. (3.20 p.m.): Animal dies with symptoms of convulsions.

Post-mortem lesions.—Ecchymoses of right endocardium; ecchymoses and extravasation of left endocardium; slight hyperaemia of lungs; *Gastrophilus* larvae present in fundus of stomach.

(7th September, 1916): *Horse* 9298, dark brown gelding, condition poor. (2.45 p.m.): Injected subcutaneously with the watery extract of 6.2 grm. (20 larvae) of *Gastrophilus pecorum* larvae (horse 9611 and horse 10434) in 20 c.c. physiological water. The extract injected amounted to 19 c.c.

(2.50 p.m.): Animal shakes the body. (2.55 p.m.): Chewing movements; looking at near-side. (3.10 p.m.): Passed a good quantity of normal faeces. (3.25 p.m.): Swishes the tail. (3.26 p.m.): Lifting the tail; rubbing the nose against the post; kicking at abdomen; slightly restless and shaking the body. (3.40 p.m.): Restlessness increased; pawing and moving from side to side; lifting the tail and defecating. (3.50 p.m.): Still very restless. (4.0 p.m.): Placed in loose box; walks round in the box but not feeding. (4.40 p.m.): Animal lies in sternal position; appears quiet; bedding attached to horse as if horse had been rolling. (6.0 p.m.): Seems to have recovered; feeding. (8th September, 1916): A somewhat painful swelling of the size of the hand has developed at the seat of inoculation. Morning temperature 100°; evening temperature 101°. (9th September, 1916): Morning temperature 102°; evening temperature 101.2°. (10th September, 1916): Swelling has formed into an abscess, which was opened during the day and discharged a yellowish, purulent, offensive-smelling material. (14th September, 1916): Discharge less; wound looks healthy; swelling going. 22nd September, 1916, i.e. on the fifteenth day, the horse was re-inoculated with the same species of larva.

(12.35 p.m.): *Horse* 9298, injected subcutaneously with the watery extract of 6.2 grm. (32 larvae) *Gastrophilus pecorum* larvae (horse 10412, 14th September, 1916) in 20 c.c. physiological water. The watery extract amounted to 20 c.c.

(12.4 p.m.): Slightly increased abdominal respiration. (12.48 p.m.): Slight chewing movements; switching the tail; pawing and shaking the head. (12.50 p.m.): Lifts the tail. (1.30 p.m.): Passes a quantity of normal faeces. (2.5 p.m.): Pawing. (2.15 p.m.): Stands fairly quiet; slightly increased abdominal respiration still present. (3.0 p.m.): Respiration normal; animal somewhat dull. (4.30 p.m.): Not feeding well. (6.0 p.m.): Appears to have recovered completely. (23rd September, 1916): At the seat of inoculation a somewhat painful swelling has developed, about the size of a hand. (25th September, 1916): Swelling has increased. (26th September, 1916): Abscess has formed—about the size of a large orange—which burst naturally, discharging a yellowish white, granular material, with a very disagreeable odour. (20th October, 1916): Horse again injected subcutaneously with 3.1 grm. *Gastrophilus pecorum* larvae of horse 10437 in 20 c.c. physiological water. On the first, second, and third days there were exacerbations of temperature to 104° F. the first day, 106.6° F. second day, and 104.8° F. on the third day, and from then onwards no other symptoms were shown. On the ninth day an abscess formed at seat of injection.

The horse was kept under observation until the 7th November, 1916, when it was again inoculated, but with *Gastrophilus nasalis* larvae to ascertain whether this horse would not show more acute symptoms, or probably die (b.f. G.9595), when injected with a different

species of larva. A small wound was still present at the seat of inoculation from the previous injection; animal had improved in condition.

(2.15 p.m.): Injected subcutaneously with the watery extract of 6.2 grm. of *Gastrophilus nasalis* larvae in 20 c.c. physiological water. Horse showed no symptoms of intoxication after the injection. (8th November, 1916): At the seat of inoculation a great swelling has formed, which extends from the base and side of the neck on to the shoulder and forearm on the same side. The swelling is hot and painful. Morning temperature 99° F.; evening temperature 104° F. (13th November, 1916): Swelling pointing on side of the neck. (14th November, 1916): Swelling has burst, discharging purulent, disagreeable-smelling material. Swelling in neck and limbs going. (25th November, 1916): Still a wound present at the seat of inoculation. Horse discharged from experiment. This horse was therefore subjected to four injections of extracts of *Gastrophilus* larvae, viz.: 7th September, 1916; 22nd September, 1916; 20th October, 1916; 7th November, 1916, and was kept under observation (temperatures daily) until the 15th December, 1916, and no symptoms of Pernicious Anaemia were detected during that time.

(6th December, 1916).—*The above experiment repeated on exactly similar lines.*

(6th December, 1916): Horse 9048, condition very poor, conjunctival mucous membranes pale. (2.30 p.m.): Injected subcutaneously with the watery extract of 6.2 grm. of *Gastrophilus pecorum* larvae (horse 10720 and horse 10750) in 20 c.c. physiological water. The filtrate amounted to 22 c.c.

(2.48 p.m.): Animal restless; defaecates; swishing the tail; biting at the sides; respiration increased in frequency. (2.50 p.m.): Respiration further increased and laboured. (2.55 p.m.): Sweating in patches; restless; pulse increased in frequency; watery discharge from the nose, in the form of drops; rubbing the head against the post. Temperature 104° F. (3.5 p.m.): Respiration laboured; moving from side to side and swishing the tail. (3.15 p.m.): Pulse very frequent; temperature 103.6° F.; shaking the head. (3.45 p.m.): Respiration shows improvement; animal standing quiet. (5.0 p.m.): Quiet and feeding. (7th December, 1916): A painful, hot swelling formed at the seat of inoculation. (11th December, 1916): The abscess which had formed at the seat of inoculation has burst, with a yellowish white and very offensive smelling discharge. (22nd December, 1916): Horse discharged from experiment. A small wound present at the site of injection.

(6th December, 1916): Horse 9646, condition very poor. (2.30 p.m.): Injected subcutaneously with the watery extract of 6.2 grm. of *Gastrophilus nasalis* larvae (horse 10720 and horse 10750), in 20 c.c. physiological water. The extract amounted to 22 c.c.

(2.40 p.m.): Animal observed to be somewhat restless; swishing the tail; shaking the body, and moving from side to side. (2.50 p.m.): Still restless; temperature 103°; pulse slightly increased in frequency; respiration slightly increased; moving from side to side. (3.5 p.m.): Passes a quantity of normal faeces. (3.15 p.m.): Respiration slightly more increased and laboured. Pawing with the front limbs. (3.25 p.m.): Temperature 101.6°; showing some improvement. (4.0 p.m.): Seems to have recovered, but is somewhat dull and not feeding well. (7th December, 1916): Feeding well; a painful, hot swelling has formed at the seat of inoculation, about the size of a hand. (13th December, 1916): The abscess, which had formed from the swelling, has burst, with a purulent, stinking discharge. (22nd December, 1916): On the sixteenth day after injection, the horse was discharged from experiment, only a small wound being still present at the site of inoculation.

(6th December, 1916): Horse 10015, chestnut gelding, condition very poor. (2.30 p.m.): Inoculated subcutaneously with the watery extract of 6.2 grm. *Gastrophilus equi* larvae (horse 10720 and horse 10750) in 20 c.c. physiological water. The extract amounted to 25 c.c.

(2.37 p.m.): Shaking the body and somewhat restless. (2.40 p.m.): Taking up a position as if wanting to defaecate. (2.50 p.m.): Moving from side to side. (2.55 p.m.): Temperature 102° F.; pulse and respiration show nothing unusual. (3.5 p.m.): Passes a small quantity of normal faeces; swishing the tail. (3.15 p.m.): Rubbing the anterior portion of the body—near-side—against a post; respiration is slightly increased. (3.25 p.m.): Temperature 103°; still somewhat restless; pulse is increased in frequency. (3.30 p.m.): Passing a small quantity of a watery faeces. (3.35 p.m.): Lifting the tail and again passing a small quantity of faeces, followed by flatus. (4.0 p.m.): Stands fairly quiet, but looks somewhat dull; pulse and respiration normal. (5.0 p.m.): Not feeding well. (7th December, 1916): Not feeding too well; painful swelling has formed at the seat of inoculation. (12th December, 1916): The abscess, which had formed, burst, discharging a disagreeable-smelling, purulent material, fairly copious. (22nd December, 1916): Discharged from experiment.

Conclusions.

1. Of the two horses, each inoculated subcutaneously with a watery extract of 6.2 grm. *Gastrophilus nasalis* larvae, one (horse 9595) showed very acute symptoms of an intoxication and died within three-quarters of an hour; the other (horse 9646) showed very slight symptoms of an intoxication and recovered within two hours.

2. Of two horses, each inoculated subcutaneously with a watery extract of 6.2 grm. of *Gastrophilus equi* larvae, one (horse 9763) showed very acute symptoms of an intoxication and died within three-quarters of an hour; the other (horse 10015) showed very slight symptoms of an intoxication and recovered within two hours.

3. Of two horses, each inoculated subcutaneously with a watery extract of 6.2 grm. *Gastrophilus pecorum* larvae, both horses (horse 9298 and horse 9048) showed slight symptoms of an intoxication and recovered within two hours.

4. One of the latter horses (horse 9298), afterwards reinjected with the same dose of *Gastrophilus pecorum* larvae showed similar symptoms.

5. The same horse (horse 9298) was still later reinoculated subcutaneously with the same dose of *Gastrophilus nasalis* larvae (i.e. the larvae which killed one horse) and showed no symptoms. From the above it would appear that there does not seem to be any appreciable difference in the toxic properties of the watery extracts made from different species of South African *Gastrophilus* larvae, viz., *pecorum*, *nasalis*, and *equi*. The different experiments carried out so far seem to bring out the fact that there exists in horses a varying susceptibility, some seeming to be more susceptible than others to the toxic action of the larvae, no matter what species is used.

2.—TRANSMISSION OF EQUINE PERNICIOUS ANAEMIA BY MEANS OF *GASTROPHILUS* LARVAE.

Seyderhelm and Seyderhelm, supported by Ries (as shown above), came to the conclusion that the *Gastrophilus* larvae are the cause of an infectious anaemia in horses. Their conclusions have recently been disproved by the further researches of Carré and Vallée, who still believe that Infectious Anaemia is due to a filterable virus, and that the toxin of the *Gastrophilus* larvae does not influence it in any way. The experiences at this laboratory are in accordance with the latter investigations, and a number of experiments have been undertaken

to prove or disprove the conclusions of Seyderhelm and Seyderhelm and others.

Ries based his views on a number of clinical observations made in the field, observations which, according to him, point to these larvae as the carriers of disease. He lays stress on the frequent occurrence of *Gastrophilus* flies in localities where equine pernicious Anaemia exists, and the presence of such larvae in the stomachs of horses that are affected with the disease. He also speaks of the frequent occurrence of a verminous aneurism of the anterior mesenteric artery in such cases.

At Onderstepoort statistics were compiled: (1) To show the frequency of *Gastrophilus* larvae in the stomachs of horses that had died at this laboratory of diseases other than pernicious anaemia during the period October, 1915, to March, 1917. The following table is given:—

1915.	GASTROPHILUS LARVAE PRESENT.			GASTROPHILUS LARVAE NOT PRESENT.		
	Good Con- dition.	Fair Con- dition.	Poor Con- dition.	Good Con- dition.	Fair Con- dition.	Poor Con- dition.
October.....	5	14	14	5	4	9
November.....	6	19	10	2	8	1
December.....	4	5	7	—	2	6
1916.						
January.....	3	35	12	—	8	3
February.....	2	11	7	—	5	2
March.....	3	22	14	2	8	8
April.....	5	4	9	1	1	5
May.....	12	28	8	—	4	3
June.....	4	10	4	—	2	1
July.....	6	24	14	1	3	3
August.....	1	7	4	3	—	—
September.....	10	2	21	1	6	3
October.....	16	14	15	1	1	—
November.....	3	7	6	1	3	1
December.....	2	3	3	—	1	2
1917.						
January.....	2	7	15	1	—	2
February.....	8	16	7	2	—	—
March.....	3	11	7	—	4	5
	95	239	177	20	60	54

From this it will be seen that 82 per cent. of the good conditioned horses, 80 per cent. of the fair conditioned horses, and 76 per cent. of the poor conditioned horses that have died at this laboratory of diseases other than pernicious anaemia have shown the presence of *Gastrophilus* larvae.

(2) To show the presence of aneurism in anterior mesenteric artery in horses that died at this laboratory of diseases other than pernicious

Anaemia during the period June, 1916, to July, 1917, the following table is given:—

1916.	ANEURISM PRESENT.			ANEURISM ABSENT.		
	Good Con- dition.	Fair Con- dition.	Poor Con- dition.	Good Con- dition.	Fair Con- dition.	Poor Con- dition.
June.....	4	4	4	2	3	1
July.....	4	10	9	1	—	—
August.....	—	4	1	—	—	1
September.....	—	3	9	2	2	—
October.....	5	3	5	—	3	—
November.....	2	6	3	—	—	—
December.....	2	1	—	—	—	—
1917.						
January.....	—	3	9	2	3	3
February.....	3	10	6	—	4	—
March.....	4	9	9	—	4	—
April.....	3	9	3	—	4	2
May.....	2	5	11	—	2	—
June.....	2	5	8	2	5	1
July.....	3	7	10	3	2	1
	34	79	87	12	32	9

From this it will be seen that 74 per cent. good conditioned horses, 71 per cent. fair conditioned horses, and 90 per cent. poor conditioned horses that have died at this laboratory of other diseases than pernicious anaemia have shown the presence of aneurisms. It appears that there does not seem to be a "Gastrophilic Anaemia" in horses in South Africa, judging from the regular occurrence of the larvae in good, fair, and poor conditioned horses. The Japanese Commission found that the removal of Bot Flies from all horses before pasturing did not have any influence on the spread of the disease. The Dutch investigators, Sohns and Soetedje, 1917, do not consider a Gastrophilic spread of equine pernicious Anaemia possible in the Netherlands India, owing to the fact that these Bots cannot exist there. The experimental evidence brought forward by the Japanese Commission with reference to the transmission of pernicious anaemia by means of watery extracts of *Gastrophilus* larvae was negative. With the experimental evidence of Seyderhelm and Seyderhelm we agree in part, viz., that the watery extracts of larvae can set up the disease when injected into horses, but equine pernicious anaemia will *only* result when such larvae are collected from horses which had been the carriers of virus during life. Evidently Seyderhelm and Seyderhelm had collected larvae for experiment from horses clinically recovered from pernicious Anaemia, and so generalized that all *Gastrophilus* larvae, immaterial of their source, could produce the disease. In another paper the author will bring forward evidence to show how such virus reservoirs in horse-sickness inoculations caused in one instance a 60 per cent. mortality. These *Gastrophilus* larvae in the

stomach of the horse evidently feed on the body fluids of the host, and, besides nourishment, they may also take in any ultravisable virus, such as that of pernicious Anaemia, that may be present in the blood. In this way, larvae experimentally become mechanical carriers of virus of diseases that may be present. That larvae do act in this particular fashion was borne out by the following experiments, in which not only pernicious Anaemia virus but also horse-sickness virus was transmitted to susceptible horses by the *Gastrophilus* larvae. In none of the horses (see previous experiments, and the following table) were any symptoms, etc., of equine pernicious anaemia observed as the result of the inoculation of watery extracts of *Gastrophilus* larvae, which had been collected from horses dead from disease *other* than pernicious Anaemia.

Date of Experiment.	No. of Horse.	Method.	Dose.	Cause of Death of Horse from which Larvae were Collected.	Subsequent History.
28. 7.15	9328	Intraj.	1 c.c. of 1 G.L.	Toxaemia	Discharged...24. 9.15 Sold..... 3. 6.16
30. 7.15	9048	Subcut.	10 c.c. of 1 G.L.	Horse-sickness	Discharged...23. 9.15
28. 7.15	9095		1 G.L.	"	" ...23. 9.15 Sold..... 5. 1.16
26. 7.15	8971		20 G.L.	"	Discharged...23. 9.15 Sold21. 1.16
28. 7.15	9019	"	40 G.L.	Toxaemia	Discharged...23. 9.15
6. 8.15	8825		60 G.L.	Horse-sickness	" ...23. 9.15
9. 8.15	8752		100 G.L.	"	" ...22. 9.15 Sold.....21. 1.16
17. 9.16	9298	Drenched	20 G.L.	"	Discharged...15.12.16
13. 8.15	8806		500 G.L.	"	" ...23. 9.15
23. 9.15	8873		1000 G.L.	Horse-sickness and debility	Sold..... 5. 1.16 Discharged... 2.11.15
6.12.16	9646	Subcut.	20 G.L.	Horse-sickness	Discharged...22.12.16

Most of these horses were kept under observation for at least ninety days, and some were retained for several months.

Experiment No. 6.—To prove that pernicious Anaemia can be produced by watery extracts of *Gastrophilus* larvae collected at post-mortems from horses which had died of pernicious Anaemia:—(6th October, 1915): *Horse* 9406, brown gelding, condition moderately fair. Conjunctival mucous membranes slightly congested. (3.13 p.m.): Injected subcutaneously with the watery extract of 20 *Gastrophilus* larvae (weight 5.7 grm.) (horse 9087, 4th October, 1915) in 20 c.c. physiological water.

History of Horse 9087.—On 6th August, 1915, horse 9087 was inoculated intrajugularly with 50 c.c. blood, a mixture of that of horse 6435, horse 6576, and horse 8564. The latter were clinically recovered cases of pernicious anaemia, which were, however, still carriers of the virus of pernicious anaemia, although they were in good condition. From the twelfth day after injection horse 9087 commenced to

show an irregular temperature reaction, which lasted until the forty-ninth day, i.e. the day of death. The fever was of an irregularly intermittent type, characteristic of pernicious anaemia. From the twenty-third day and onwards, the frequency of the pulse increased. On the twenty-first day the near hind limb was observed to be swollen; on the twenty-third day there was an oedematous swelling under the abdomen; on the twenty-fifth day the animal commenced to drag its hind limbs, ecchymoses were present on the conjunctival mucous membranes; on the twenty-sixth and twenty-ninth days the swelling under the abdomen had increased; on the thirty-first day an orange tinge of the conjunctival mucous membranes, with ecchymoses was present; on the forty-ninth day it died of pernicious anaemia. The blood of horse 9087 was controlled in horse 9381 on 16th September, 1915. The latter also contracted pernicious anaemia, followed by a reaction to Nuttalliosis. It died of pernicious Anaemia and Nuttalliosis. (3.13 p.m.): Horse 9406 injected.

(3.14 p.m.): Small quantities of dry faeces passed; pronounced chewing movements. (3.16 p.m.): Stamping with legs and pawing with front limbs; raising tail and kicking at the belly several times; tail remains raised. (3.17 p.m.): Passed small quantity of faeces; tail still raised; rubbing the nose against the pole. (3.18 p.m.): Standing with legs spread out, as if wanting to stale; swishing the tail; does not pass any urine. (3.19 p.m.): Shaking the head; chewing movements; again assumes position as if attempting to micturate. (3.27 p.m.): Kicking at the abdomen. (3.30 p.m.): Respiration increased and laboured; drops of perspiration trickling down from inside of thigh and perineum. (3.35 p.m.): Animal standing fairly quiet; perspiration more profuse, and is also present on the nose, quarters, neck, and sides of the body. (3.40 p.m.): Respiration 34 and laboured; passes a small quantity of faeces, softer in consistence. (3.41 p.m.): Passes a quantity of watery faeces, with a somewhat offensive odour. (3.43 p.m.): Ditto. (3.52 p.m.): Ditto. (3.55 p.m.): Respiration 20, not so laboured; perspiration much less; animal placed in a loose-box. (4.0 p.m.): Walking round in the box; making attempts to lie down; respiration still slightly increased. (4.30 p.m.): Lying in sternal position; distinctly dull, but quite quiet. (6.0 p.m.): Still found lying in sternal position; horse has not made any attempts to feed. (7th October, 1915): Feeding; a hot painful swelling of the size of a hand found at the seat of inoculation. (8th October, 1915): Morning temperature 100° F.; evening temperature 102° F. (11th October, 1915): Swelling at seat of inoculation has burst, a yellowish white, granular discharge, with a very disagreeable odour escaping from the wound. (19th October, 1915): Still a slight discharge present; swelling has greatly decreased in size. (20th October, 1915): From this day, i.e. the fourteenth day after injection, the horse begins to show an irregular reaction, with irregularly intermittent fever up to the date of death, i.e. the thirty-fifth day (see chart). (27th day): Conjunctival mucous membrane has a slight orange tinge and is somewhat dirty. (29th day): Animal not steady in its hindquarters. (32nd day): Not feeding well; tucked up; respiration 18; pulse 64; conjunctival mucous membrane dark red; ataxia, which makes the gait clumsy and staggering. (33rd day): Falling off in condition rapidly; not feeding; haemorrhagic discharge from nostrils and conjunctival mucous membrane; ataxia more marked; staggers from side to side; pulse 84; respiration 22. (34th day): Horse weak; staggering and swaying more marked; moves with great difficulty and uncertainty; haemorrhagic discharge still present; pulse 100; died during the night of acute pernicious anaemia.

Post-mortem lesions.—Haemorrhagic infarcts in both lungs; hydropericardium; ecchymoses of epicardium, and left endocardium; ecchymoses of serosa; tumour splenis; ecchymoses in small and large intestines; gastrophilus larvae; lower end of septum nasi few petechiae; ecchymoses on conjunctival mucous membranes. Thrombosis of anterior mesenteric artery; anaemia.

The blood of horse 9406 was controlled in horse 9433, which died of acute pernicious anaemia. (8th October, 1915): Horse 9002, grey gelding, condition fair, respiration 14, pulse 52. (2.42 p.m.): Inoculated subcutaneously with the watery extract of 20 *Gastrophilus* larvae

(weight 7.5 grm.) (horse 9381, 1st October, 1915) and 20 c.c. physiological water.

(For history of horse 9381, see previous experiment.)

(2.42 p.m.): Horse 9002 injected. (2.44 p.m.): Swishing the tail and shaking the head; tail slightly raised. (2.49 p.m.): Tail slightly raised; looking at the sides; shaking head; lifting the legs alternately; somewhat restless. (2.51 p.m.): Tail still raised; passing balls of faeces; shaking the body and looking round at the sides frequently. (2.55 p.m.): Shaking the head and rubbing its nose against the pole. (2.56 p.m.): Kicking several times at the abdomen. (2.58 p.m.): Chewing movements; lifting the tail and taking up a position as if wanting to stale; swishing the tail and finally passing a quantity of yellowish turbid urine. (3.3 p.m.): Animal more restless; rubbing the nose persistently against the front limbs; pawing with front limbs; increased and laboured respiration; chewing movements. (3.10 p.m.): Tail still kept raised; rubbing the nose. (3.20 p.m.): Restless; shaking the head; lifting the legs alternatively, and kicking at the belly with the hind limbs; rubbing the nose. (3.30 p.m.): Animal appears quieter; respiration 24. (4.30 p.m.): Animal seems to have recovered; feeding; temperature 102.4° F. (9th October, 1915): Morning temperature 101° F.; evening temperature 102° F.; painful, hot-swelling about the size of two hands formed at the seat of inoculation. (11th October, 1915): Swelling slightly increased; still slight temperature. (12th October, 1915): Swelling burst, with a yellowish white granular discharge from the abscess, with a very disagreeable odour. (19th October, 1915): Swelling decreased; slight discharge present. From the twenty-fourth day, i.e. 1st November, 1915, horse commences to show temperature reaction of pernicious Anaemia, with five distinct remissions of temperature:—

- (1) From the twenty-fourth to the thirty-second day fairly marked. Twenty-fourth day, 104° F. Twenty-fifth day, 103.8° F. and 105° F. Twenty-sixth day, 101° F. and 104° F. Twenty-seventh day, 99.6° F. and 100° F. Also a slight swaying movement in the hindquarters; slight ecchymoses of conjunctival mucous membranes. Twenty-ninth day, 99.8° F. and 103° F. Thirtieth day, 102° F. and 103° F.; hind limbs slightly swollen. Thirty-first day, 101.4° F. and 100.6° F.; dragging hind limbs slightly.
- (2) From thirty-eighth to forty-seventh day fairly marked. Thirty-ninth day, 102.4° F. and 104° F. Fortieth day, 104.4° F. and 106° F. Forty-first day, 104° F. and 104° F. Forty-third and forty-fourth days, dragging hind limbs.
- (3) From ninetieth day to ninety-fifth day, less marked; maximum temperature, 105° F. on ninety-third day.
- (4) From one hundred and thirty-fifth to one hundred and thirty-eighth days, less marked; maximum temperature, 105.4° F. on one hundred and thirty-fifth day.
- (5) From one hundred and forty-ninth day to one hundred and fifty-fifth day, less marked; maximum temperature, 104.6° F. on one hundred and fifty-third day. Followed by slight exacerbations of temperature every now and then. Animal discharged from experiment on 25th April, 1916.

Conclusions.—(1) That no cases of pernicious anaemia were observed in horses into which *Gastrophilus* larvae extracts were injected, when these were collected from horses dead of diseases other than pernicious anaemia. (2) That in both horses injected with *Gastrophilus* larvae extracts, which were collected from horses which died of pernicious anaemia, this disease resulted—acute in horse 9406 and chronic in horse 9002.

Experiment No. 7.—To ascertain whether the watery extracts of *Gastrophilus* larvae collected at post-mortems from horse-sickness carcasses could produce horse-sickness when injected into susceptible horses. (21st March, 1916): Horse 9556, grey gelding, condition very poor. (3.25 p.m.): Injected subcutaneously with the watery extract of 20 *Gastrophilus* larvae (horse 10915) in 20 c.c. physiological water.

(*History of Horse 10915*: On 15th March, 1916, injected subcutaneously with 2 c.c. virulent horse-sickness blood, viz., virus horse 9866 O.179. gen. It developed horse-sickness and died on the 6th day after injection.)

(3.35 p.m.): Slight masticatory movements. (3.50 p.m.): Passed a fair quantity of normal faeces. (3.55 p.m.): Animal standing quiet; somewhat dull. (4.30 p.m.): Lying in sternal position; slightly increased respiration. (22nd March, 1916): Animal feeding well. (25th March, 1916): Evening temperature 105° F.; animal very weak; abscess formed at the seat of inoculation. (26th March, 1916): Died during the day; cause of death assigned to debility and poverty. However, the blood of this horse was not further tested for horse-sickness.

(18th July, 1916): *Horse 10364*, grey gelding, condition fair, conjunctival mucous membrane slightly injected, pulse good. (11.40 a.m.): Injected subcutaneously with the watery extract of 20 *Gastrophilus* larvae (horse 10206) in 20 c.c. physiological water. (*History of Horse 10206*: Injected subcutaneously on 9th July, 1916, with 2 c.c. virulent horse-sickness blood, viz., virus horse 10299 O. 181 gen. From the fourth day there was a reaction to horse-sickness, with death from horse-sickness on the night of the sixth day.)

(11.48 a.m.): Animal somewhat restless and shakes the body several times; from then until 12.30 p.m. frequently observed to yawn, with licking and chewing movements; rubbing the nose and head against a post; slightly increased abdominal respiration. (12.45 p.m.): Animal observed to feed. (2.0 p.m.): Horse recovered completely. (19th July, 1916): Slight swelling has formed at the seat of inoculation, hot and somewhat painful; also oedematous swelling of sternal region. (22nd July, 1916): Abscess, which had formed at the seat of injection, burst, discharging a very offensive white granular material. From this day the horse commenced to show a horse-sickness reaction with remittent fever. Morning temperature 105° F.; evening temperature 106.6° F. (23rd July, 1916): Morning temperature 101° F.; evening temperature 105° F. (24th July, 1916): Morning temperature 105° F., followed by collapse; horse died during the night of horse-sickness.

Post-mortem lesions.—Hydropericardium; hyperaemia and oedema of both lungs; ecchymoses of left endocardium; hyperaemia of kidneys, fundus of stomach, and intestines; tumour splenis.

(27th July, 1917): *Horse 11384* (in horse-sickness filtrate experiment) was injected subcutaneously with the watery extract of 30 *Gastrophilus* larvae (collected from horse 11385 and horse 10890, which had died of horse-sickness) in 30 c.c. water. Horse showed symptoms of an intoxication which lasted about two hours. (1st August, 1917): Evening temperature, 102.4° F. (2nd August, 1917): Morning temperature, 103.6° F.; horse went down during the night. (3rd August, 1917): Morning temperature 103° F.; horse died during the day. Post-mortem showed "horse-sickness."

Conclusions.—Of three horses susceptible to horse-sickness injected with the watery extracts of *Gastrophilus* larvae collected from horses dead of horse-sickness, two died of horse-sickness, and the other horse died of what was suspected as senility and poverty, but its blood was not tested to exclude horse-sickness.

Experiment No. 8.—To ascertain whether the filtrate (through a Berkefeld) from the watery extract of *Gastrophilus* larvae collected at post-mortem from a horse dead of pernicious anaemia could produce the disease. (7th December, 1915): *Horse 9772*, grey gelding, condition poor, injected subcutaneously with 50 c.c. filtrate of

the watery extract of 20 *Gastrophilus* larvae (horse 9443, 23rd November, 1915) in 200 putrid water (through a Berkefeld filter candle). [*History of Horse 9443* (see above): It died of acute pernicious anaemia on the fourteenth day after injection of 50 c.c. blood (horse 9406), also suffering from pernicious anaemia.]

Horse 9772 showed no symptoms of an intoxication after the injection of the filtrate and no swelling developed at the seat of inoculation. This animal gradually fell off in condition and showed frequent exacerbations of temperature, and slight remissions. (31st day): Morning temperature 99.2° F.; evening temperature 103.6° F. (32nd day): Morning temperature 102° F.; evening temperature 100.4° F.; pulse 48; conjunctival mucous membrane pale. (38th day): Morning temperature 99° F.; evening temperature 102.6° F. (60th to 65th day): A reaction; maximum temperature 103° F. (87th to 94th day): A reaction; maximum temperature 103.6° F. (102nd to 112th day): A reaction; maximum temperature 103.6° F. (126th to 137th day): A reaction; maximum temperature 103.2° F.; pulse frequency during this period varied between 56 and 92 per minute. Horse also showed a pale and somewhat watery conjunctival mucous membrane, with a tinge of orange; animal had markedly lost in condition; dragging its hindlegs; a very cachectic general appearance; a few days later a few superficial ecchymoses observed on the membrana nictitans. (180th day): Another exacerbation of morning temperature 101.7° F.; evening temperature 102° F. Animal extremely poor in condition; hind limbs swollen; disinclined to walk; had been down during the night and showed abrasions on both sides of the shoulder, hip, and knee. It was killed on the 193rd day in extremis.

Post-mortem lesions.—Anaemia; atrophy of liver and spleen; slight hypuraemia of fundus (stomach). It appears that this horse showed symptoms resembling pernicious anaemia, but no definite conclusions could be drawn. It was therefore decided to control this horse's blood.

On 28th March, 1916, *Horse 8756* received intrajugularly 50 c.c. defibrinated blood (horse 9772). *Horse 8756* was in rather poor condition at the time.

From the seventh day the horse began to show an irregular reaction, with irregular remissions of fever, somewhat characteristic of pernicious anaemia. It died on the fifty-ninth day after injection. High evening temperatures were recorded during this period, viz., 103.6° F. on the eleventh day; 103.4° F. on the twelfth day; 103° F. on the twenty-seventh day; and 104° F. on the twenty-ninth day. (32nd day): Morning temperature 99° F.; evening temperature 102.4° F.; pulse 52; conjunctival mucous membrane pale, with an orange tinge and slight ecchymoses on the membrana nictitans. (59th day): Was found down in the morning; unable to rise, and unable to stand when lifted; it died during the day. Since injection it had markedly fallen off in condition.

Post-mortem lesions.—Anaemia; icterus; hydropericardium, ecchymoses of epicardium, and both endocardiums; degeneration of liver; ecchymoses in intestines; lobulation of liver not distinct; malpighian bodies of spleen enlarged; *Gastrophilus* larvae present in stomach and duodenum.

Even here no definite conclusions could be drawn, and it was decided to repeat the whole experiment.

(24th January, 1917): *Horse 10891*, stallion, good condition. Injected subcutaneously with 200 c.c. filtrate (through Berkefeld filter) of a watery extract of 45 *Gastrophilus* larvae (horse 9957, 22nd January, 1917) in 450 c.c. water. [*History of Horse 9957*: Grey gelding, condition rather poor; on 7th October, 1916, it was injected intrajugularly with 50 c.c. blood (horse 6435), a clinically recovered case of pernicious anaemia. From about the sixteenth day it commenced to show an irregular temperature reaction, with exacerbations of temperature never exceeding 103° F. From about the fiftieth day there was slight intermittent fever right up to the time of death, never exceeding 102° F. The animal was also losing in condition rapidly, and during the last ten days it showed acute swelling of the hind limbs, front

limbs, and sternal region, which gradually increased; conjunctival mucous membrane was pale, with ecchymoses; dragging of the hind limbs and swaying gait became more marked, and the animal was inclined to stumble. Eventually the horse went down and was unable to rise. It died of pernicious anaemia on the seventy-sixth day after infection.] *Horse 10891* showed no symptoms of intoxication after injection, nor did any swelling form at the seat of injection. The horse was temperatured daily and kept under observation for more than six months, and no symptoms of pernicious anaemia were observed.

(17th August, 1917): *Horse 11361*, bay gelding, condition fair, injected subcutaneously with 275 c.c. filtrate (through a Berkefeld filter) of the watery extract of 22 *Gastrophilus* larvae (*horse 11029*) in 1000 c.c. physiological water. (*History of Horse 11029*: On the 12th February, 1917, injected intrajugularly with 5 c.c. pernicious anaemia blood from horse 10913. From the fifteenth day after injection the horse commenced to show a reaction to pernicious anaemia, with death on the thirtieth day.)

No symptoms of an intoxication were observed up to the twenty-first day, on the 6th September, 1917, i.e. on the twenty-first day, horse 11361 was reinjected subcutaneously with 250 c.c. filtrate (through a Berkefeld filter) of the watery extract of 25 *Gastrophilus* larvae (*horse 11001*) in 500 c.c. water. (*History of Horse 11001*: On the 20th August, 1917, horse admitted, showing symptoms of pernicious anaemia, and died on 25th August, 1917.) No symptoms of an intoxication were observed. (27th September, 1917): From this day onwards horse commenced to show an irregular fever reaction (see chart). (5th October, 1917): Symptoms of pernicious Anaemia became evident. Loss of condition; conjunctival mucous membrane showed an orange tinge; with slight hyperaemia. (7th October, 1917): Mucous membrane showed ecchymoses, which became more marked on 9th October, 1917. There was also an increase in the frequency of the pulse. (11th October, 1917): Blood-smear gave negative result. (12th October, 1917): Swellings of the limbs were observed, with the mucous membranes haemorrhagic. (16th October, 1917): Animal was destroyed for post-mortem.

Pathological anatomical diagnosis.—Anaemia; haemorrhagic infarcts in both lungs; slight tumour splenis; degeneration in liver, kidneys, and myocardium; slight hyperaemia in stomach and intestines; majority of lymphatic glands swollen, oedematous, and slightly hyperaemic.

Conclusions.—Of three horses injected subcutaneously with the filtrate (through a Berkefeld filter) from the watery extract of *Gastrophilus* larvae, collected at post-mortems from horses which died of pernicious anaemia, (a) horse 10891 showed no symptoms. (That this sometimes does happen will further be explained in another paper on pernicious anaemia by the author.) (b) Horse 9772 showed somewhat doubtful symptoms (the control horse injected with its blood also showed doubtful symptoms). (c) Horse 11361 showed definite symptoms of equine pernicious anaemia.

Experiment No. 9.—To see whether a watery extract of *Gastrophilus* flies, hatched from pupae coming from pernicious Anaemia horses can produce (a) symptoms of an intoxication; (b) pernicious Anaemia.

(8th June, 1916): *Horse 9315*, dark brown gelding, condition poor, injected subcutaneously with a watery extract of nine *Gastrophilus* flies (eight males and one female). These flies were bred out from larvae collected during the season 1915-1916 from three clinically recovered pernicious Anaemia horses, viz., horse 6435, horse 6576,

and horse 8564. These horses subsequently proved to be still virus carriers. The flies were preserved in about 30 c.c. of 25 per cent. glycerine. The whole was pounded up in a mortar and passed through muslin; to the filtrate 70 c.c. physiological water was added and the resulting mixture inoculated. There were no symptoms present after the injection. About five hours afterwards a swelling of about the size of a hand, hot and painful, had formed at the seat of inoculation. It soon disappeared. This horse was temperatured daily until discharged from experiment on 29th September, 1916. There were no signs of pernicious Anaemia. No conclusions could be drawn from the foregoing experiment. A sufficient number of pupae could not be collected from the droppings of pernicious Anaemia horses; evidently these animals were not badly affected, because they were stabled for the greater part of the year. No further experiments could be carried out, as no adult flies bred from pupae were available.

Experiment No. 10.—To ascertain whether the filtrate (through a Berkefeld filter) from the watery extract of *Gastrophilus* larvae collected at post-mortems from horses that died of horse-sickness could produce horse-sickness in susceptible horses. (7th September, 1916): *Horse* 10450, bay gelding, condition poor. (3.30 p.m.): Injected subcutaneously with 20 c.c. filtrate (through a Berkefeld filter) of the watery extract of 20 *Gastrophilus nasalis* larvae, 20 *Gastrophilus equi* larvae, 20 *Gastrophilus pecorum* larvae, in 100 c.c. water. The larvae were collected from horses 9611 and 10434, both of which had died of horse-sickness.

(3.45 p.m.): Animal blowing slightly. (4.0 p.m.): Not feeding; slight abdominal respiration; a quantity of soft faeces passed. (6.0 p.m.): Animal lying in the sternal position, having taken very little food; dull. (8th September, 1916): No swelling observed at the seat of inoculation. It was discharged from experiment after twenty-six days, and during that period showed no symptoms of horse-sickness.

(3rd October, 1916): The horse was inoculated with a virulent horse-sickness virus (horse 10206 O. 182 gen., 14th July, 1916) to ascertain whether it was a susceptible or immune horse. It, however, promptly reacted to the virus, and died of horse-sickness on the sixth day after injection.

(7th November, 1916): *Horse* 10683, grey gelding, at 3.10 p.m. injected subcutaneously with 100 c.c. filtrate of watery extract of 25 *Gastrophilus equi* larvae and 25 *Gastrophilus nasalis* larvae (horse 10447, 5th November, 1916, in 300 c.c. water. (*History of Horse* 10447: Immunized against horse-sickness on 17th October, 1916. On the nineteenth day after injection it died of Dikkop.)

(3.25 p.m.): *Horse* 10683 appeared somewhat restless; observed to defecate frequently; faeces of a slightly watery consistence; slightly increased abdominal respiration. (4.30 p.m.): Slight expiratory dyspnoea; still passing watery faeces, which emit a disagreeable odour. Animal still restless and depressed; walked round in the box and then lay down in sternal position, but immediately rose again. This was repeated several times. (6.0 p.m.): Animal standing quiet, but somewhat dull, and not feeding well. (8th November, 1916): Animal recovered completely and feeding well. It was discharged from experiment on the fifteenth day, no symptoms of horse-sickness having been observed.

(2nd November, 1916): It was inoculated with a virulent horse-sickness virus (horse 10699, O. 176 gen., 13th November, 1916) to

ascertain whether it was a susceptible or an immune horse. It, however, promptly reacted to the virus, and died of horse-sickness on the seventh day after injection.

(13th July, 1917): *Horse 11387*, bay gelding, condition poor. (12.0 p.m.): Injected subcutaneously with 250 c.c. filtrate (through a Berkefeld filter) of a watery extract of 100 *Gastrophilus* larvae (horse 11330 and horse 11359) in 1000 c.c. water. (*History of Horse 11330*: Injected with a virulent horse-sickness virus on 20th June, 1917. It developed horse-sickness and died on the twelfth day after injection. *History of Horse 11359*: Injected with a virulent horse-sickness virus on 20th June, 1917. It developed horse-sickness and died on the thirteenth day after injection).

Five minutes after injection the horse became distinctly restless; swayed from side to side; dyspnoea and profuse perspiration. (2.0 p.m.): Passing watery foetid faeces frequently; respiration increased and abdominal; animal still restless. (3.0 p.m.): Lying in sternal position; distinctly dull; diarrhoea still present. (4.0 p.m.): Not feeding; standing with head hanging and listless. Horse recovered completely; no swelling formed at seat of the injection.

(27th July, 1917): Horse had not yet shown any symptoms of horse-sickness. It was decided to inject it with the watery extract of *Gastrophilus* larvae collected from horses that died of horse-sickness to ascertain (a) whether the horse in question is a susceptible or an immune horse; (b) if the extract can transmit horse-sickness, as shown in a previous experiment.

(2.25 p.m.): *Horse 11384* was injected subcutaneously with the watery extract of 30 *Gastrophilus* larvae (horse 11385 and horse 10890, for their respective histories see the following experiment) in 30 c.c. water.

(2.30 p.m.): Horse somewhat restless; moves from side to side; tail raised; defecates. (2.35 p.m.): Yawns; stands fairly quiet; slightly increased abdominal respiration; patches of respiration noticed on the quarters; animal defecates. (2.40 p.m.): Drops of clear fluid from both nostrils; chewing and licking movements; horse moves from side to side; again defecates. (3.0 p.m.): Animal stands fairly quiet; appears somewhat dull; respiration still increased. (3.30 p.m.): Stands quiet; placed in a loose box; does not feed. (28th July, 1917): Horse recovered completely; a painful swelling of about the size of a hand formed at the seat of injection. (1st August, 1917): Evening temperature 102.4° F. (2nd August, 1917): Morning temperature 103.6° F.; evening temperature 104° F.; horse went down during the night; abscess which had formed at the seat of inoculation burst, discharging a putrid, yellowish white material. (3rd August, 1917): Morning temperature 103° F.; horse dies during the day. Post-mortem shows horse-sickness.

(27th July, 1917): *Horse 11405*, injected subcutaneously with 250 c.c. filtrate (through a Berkefeld filter) from the watery extract of 150 *Gastrophilus* larvae (horse 11385, horse 10890, and horse 11020, all of which had died of horse-sickness) in 3000 c.c. water. Horse showed no symptoms of an intoxication after injection. No symptoms of horse-sickness were observed until 17th August, 1917, i.e. the twenty-first day, when it was reinjected subcutaneously with 260 c.c. (filtrate through Berkefeld filter) from the watery extract of 30 *Gastrophilus* larvae (horse 11401) in 1200 c.c. water. (Horse 11401 had died of horse-sickness.) Horse showed no symptoms of an intoxication after injection. No symptoms of horse-sickness were observed until 11th September, 1917, when horse was injected intrajugularly with 5 c.c. virulent horse-sickness virus (horse 11402 (Tz. 17 gen.) to ascertain

whether the horse was a susceptible or an immune animal. It gave no reaction to horse-sickness, and the horse was regarded as immune.

(Experiment repeated on 25th September, 1917): *Horse 11382*, condition fair. (12.30 p.m.): Injected subcutaneously with 250 c.c. filtrate (through a Berkefeld filter) from the watery extract of 40 *Gastrophilus* larvae (*horse 11442*) in 1000 c.c. water. (*History of Horse 11442*: Died of acute horse-sickness.)

(2.30 p.m.): Horse restless; increased abdominal respiration; anxious expression of the face; animal sways slightly; paws with forelegs; muscular tremors. (4.0 p.m.): Stands fairly quiet, but appears somewhat dull; observed to have passed a quantity of soft faeces. (26th September, 1917): Horse had recovered completely. The horse showed no symptoms of intoxication. (9th October, 1917): It was decided to inject the horse with the same virus which killed horse 11442 (see above), i.e. virus horse 11402 Tz. 17th generation, to ascertain whether the animal was immune or susceptible. (14th October, 1917): Horse commenced to show a slight reaction to horse-sickness, with remittent fever, which lasted up to the 23rd October, 1917, showing that horse 11382 was susceptible to horse-sickness at the time it was injected with the filtrate.

Discussion.—Of five horses injected with the filtrate (through a Berkefeld filter) of the watery extract of *Gastrophilus* larvae from horses dead of horse-sickness, none contracted the disease. One of the five horses proved to be an immune horse, whereas the rest all showed horse-sickness to the subsequent injection of virulent horse-sickness blood. The injections were made with doses of filtrate varying from 20 c.c. to 250 c.c. Very likely the horse-sickness virus remains behind and becomes fixed to one or other non-filter-passing substances present in the extract.

GENERAL CONCLUSIONS.

1. *Gastrophilus* larvae contain a toxin, which produces definite symptoms of intoxication, etc., when extracted and injected subcutaneously, intrajugularly, and by drenching into horses, and injected intrajugularly into mules, donkeys, sheep, and goats.

2. The horse seems to be the most susceptible to the toxin, but even here there is a varying degree of susceptibility.

3. The toxin of the *Gastrophilus* larvae when extracted passes through a Berkefeld filter.

4. No symptoms of an intoxication were observed in cattle, dogs, rabbits, and guinea-pigs; the symptoms, etc., observed in rabbits being due to infectious germs injected with the non-sterile watery extracts.

5. In view of our present knowledge on Anaphylaxis there does not seem to be sufficient evidence on hand to explain the symptoms, etc., observed in horses, etc., on the lines of an Anaphylaxis.

6. There does not seem to be a relative difference in the toxicity of the extracts made from the three different species of larvae which occur in South Africa, viz., *Gastrophilus nasalis*, *Gastrophilus equi*, and *Gastrophilus pecorum*.

7. About 80 per cent. of the horses that died of diseases other than pernicious Anaemia showed the presence of *Gastrophilus* larvae, and aneurism of the anterior mesenteric artery.

8. Watery extracts of *Gastrophilus* larvae taken from horses that died of diseases other than pernicious Anaemia cannot produce pernicious Anaemia when injected into susceptible horses.

9. Watery extracts and filtrates (through a Berkefeld filter) of *Gastrophilus* larvae taken from horses dead of pernicious Anaemia can produce the disease when injected into susceptible horses.

10. *Watery extracts* of *Gastrophilus* taken from horses dead of horse-sickness can produce horse-sickness when injected into susceptible horses.

11. Of four susceptible horses injected with the *filtrate* (through a Berkefeld filter) of *Gastrophilus* larvae taken from horses that died of horse-sickness none contracted horse-sickness.

ADDENDUM.

The author regrets that up to the present he has only had access to an extract of Seyderhelm and Seyderhelm's paper (on the Aetiology of Equine Pernicious Anaemia) as it appeared in the *Schweiz. Archiv. f. Tierheilkunde*. Owing to hostilities in Europe the original paper arrived in South Africa in March, 1918, in spite of the fact that frequent attempts had previously been made to obtain a copy. The present paper of the author has to be forwarded to the Press, so a number of points brought forward by Seyderhelm and Seyderhelm will be more fully discussed in a further paper on equine pernicious anaemia as it occurs in South Africa.

1. At present we are not in a position to state how horses contract this disease naturally. We suspect "fly" transmission, and a number of experiments are being undertaken in this connection, but at present no conclusion can be drawn from them. There is no evidence to show that infection from horse to horse occurs through the medium of contaminated fodder, in spite of the fact that infected horses are frequently stabled in close contact with healthy horses. Moreover, no epidemic at pasture, described as occurring in America, Japan, France, Germany, etc., has been experienced in South Africa, yet 80 to 90 per cent. of the horses at pasture are heavily infected with *Gastrophilus* larvae. The author does not think that the evidence brought forward by Seyderhelm and Seyderhelm yet explains how it is that only a certain number of horses infected with *Gastrophilus* larvae contract equine infectious anaemia, viz.:—

(a) Idiosyncrasy as it affects the horse. In the above paper the author drew attention to a variable susceptibility of the horse to the toxin of the *Gastrophilus* larvae, but there is no such idiosyncrasy in the horse where it relates to the virus of pernicious Anaemia. The experience at this laboratory has been that if a horse is not a virus carrier of pernicious Anaemia, it can be infected by inoculation with one or the other virulent pernicious anaemia viruses.

(b) Variable toxicity of different *Gastrophilus* larvae of the same species, and of different species. This the author was not in a position to establish, and from the experiments quoted by Seyderhelm and Seyderhelm, viz., horse 9, horse 19, and horse 17, the author fails to understand how they could draw their conclusions as regards such a variable toxicity. Finally, the evidence, if correct, brought forward by Smit in 1917, would be conclusive in proving that *Gastrophilus* larvae cannot be considered as the cause of equine infectious anaemia. Dr. Smit in a paper describing the presence of

Gastrophilus larvae in an imported horse from China, points out that these larvae do not occur in the Netherlands East Indies, and that the flies cannot thrive there, in spite of the fact that attempts have been made to hatch them. Sohns and Soetedje mention in their paper on equine pernicious anaemia in 1917 that native horses contracted the disease naturally, and they do not consider *Gastrophilus* larvae as a cause of the disease on account of the above experience of Smit and others.

2. As pointed out by the author, toxic symptoms, etc., and in some instances death, were produced in the horse by the injection of extracts, etc., of *Gastrophilus* larvae, but in no instance was there any anaemia or secondary fever observed, and in none of the post-mortems were such acute lesions as congestion of all organs, great enlargement of the spleen (measurements are not given), numerous haemorrhages in kidneys, extravasations in subserosa, etc., observed, as described by Seyderhelm and Seyderhelm in horse 1, horse 10, and horse 19, etc. Secondary fever, i.e. as the result of pernicious anaemia, was produced in healthy horses by extracts of *Gastrophilus* larvae *only* when such larvae were collected from horses that died of pernicious anaemia. In the same way secondary fever, i.e. as the result of horse-sickness, was produced in healthy horses by extracts of *Gastrophilus* larvae *only* when such larvae were collected from horses that died of horse-sickness, where in each case the virus of the disease was responsible for the fever. These experiments were carried out subcutaneously, and in all cases very acute abscesses formed showing that the extracts of the *Gastrophilus* larvae contained some pathogenic organisms. Moreover, it was found that the mortality in rabbits with such non-sterile extracts in sufficiently large doses was 100 per cent., the rabbits dying of a septicaemia in from eighteen hours to several days. That the toxin of *Gastrophilus* larvae was not responsible for this mortality was further shown when the filtrate through a Berkefeld filter produced typical symptoms of an intoxication and death when injected into a horse, whereas no symptoms or deaths occurred in rabbits injected with the same filtrate. Seyderhelm and Seyderhelm have not brought forward evidence to show that the disease produced by them was perhaps the result of introducing some organism with the extract, which may become very pathogenic for the horse when injected intravenously. They state that heating ensures sterilization, but no experiments are given (on page 67) to prove this fact.

3. It has not yet been settled that internal parasites secrete or excrete toxins (which toxins produce symptoms when extracted from parasites and injected into the animal) in the living host, and the one experiment quoted by them does not prove this fact, viz., 10 *Gastrophilus* larvae were left for five days in 25 c.c. Ringers solution; the larvae remained alive, and the liquid within which they were suspended became opaque; the liquid finally was filtered and injected into horse 38, and within fifteen minutes spasms and injected mucous membranes were noted, which lasted about two hours. Again a non-sterile extract was injected, and accidental organisms cannot be excluded. Such a picture as described by them, "symptoms of shock," as we call it, is of frequent occurrence at this laboratory, where it may be observed in some of the horses injected with anti-horse-sickness serum, certain drugs, such as arrenhal, etc.

4. Finally three horses (Nos. 40, 32, and 18) eventually produce a disease like equine pernicious Anaemia by repeated injections of sub-lethal doses of extract of *Gastrophilus* larvae; the reactions agree in all three horses. In the intervals between the injections there are exacerbations of temperatures, fall in number of blood corpuscles, and haemoglobin content, and from a certain point onwards the anaemia progresses without further injections, subsequently leading to death of the animals. The pathological-anatomical picture (given on page 69) resembled that of pernicious Anaemia. They were also able to transmit this disease from the above horses to horses 31 and 35 by blood inoculations, and again from horses 31 and 35 to horse 39 by blood inoculation. For the following reasons they conclude that *Gastrophilus* larvae are the cause of pernicious anaemia:—(1) In all cases of pernicious anaemia they found *Gastrophilus* larvae in the stomach (not seen in P. A. horse 11103 experimentally infected at this laboratory). (2) By repeated injections of a specific toxic substance obtained from *Gastrophilus* larvae they produced a fatal disease in horses with acute anaemia, clinically, pathologically, and especially histologically, resembling equine infectious anaemia. (3) This disease so produced can be transmitted to healthy horses by blood inoculation.

We cannot accept the above for the following reasons:—

(1) We were unable to produce such conditions in any of the horses treated with injections of *Gastrophilus* larvae extracts, and in doses much larger than those used by Seyderhelm and Seyderhelm. D.O.B. horse 9298 showed no symptoms, although it was injected four times as follows:—7th September, 1916: Injected subcutaneously with the watery extract of 6.2 grm. (20 larvae) of *Gastrophilus* larvae in 20 c.c. physiological water, causing slight symptoms of an intoxication. 22nd September, 1916: Injected subcutaneously with the watery extract of 6.2 grm. (32 larvae) of *Gastrophilus* larvae in 20 c.c. physiological water, causing doubtful symptoms of an intoxication. 20th October, 1916: Injected subcutaneously with the watery extract of 3.1 grm. of *Gastrophilus* larvae in 20 c.c. of physiological water; no symptoms. 7th November, 1916: Injected subcutaneously with the watery extract of 6.2 grm. of *Gastrophilus* larvae in 20 c.c. of physiological water.

(2) As Seyderhelm and Seyderhelm correctly point out, there is no analogy to prove that a toxic substance can produce a disease in an animal which can be transmitted to other animals by blood inoculation. This chemical substance in horses 31 and 35 would have become so diluted in the blood of the animals that it becomes a negligible quantity, and so we must assume that the toxin multiplies in the horse in order to produce the disease again in horse 39, i.e. the inanimate becomes animate. Such a position would revolutionize all the fundamental laws of the biological world.

(3) According to Seyderhelm and Seyderhelm, it appears that the mortality of pernicious anaemia in horses is 100 per cent., and with this we cannot agree. According to our own experience it ranges between 60, 70, and 80 per cent.: a number of horses recover clinically to such an extent in some cases that without a previous history of the animal we cannot diagnose the disease except by blood inoculation. Such horses can harbour the virus for years. Some horses at this

laboratory showed their last fever reaction in 1914, although their blood was still infective at the end of 1917. In a number of horses infected with virulent pernicious anaemia virus, only one or two remissions of temperature were shown, without any other clinical symptoms. That such horses were infected was subsequently proved by blood inoculation. Such cases at pasture would easily escape the notice of the clinician, therefore, it is a pity that Seyderhelm and Seyderhelm do not state whether it was known whether the larvae used for the injection of horses 40, 32, and 18 were collected from horses free from pernicious Anaemia virus or whether horses 40, 32, and 18 were free from such a virus, because then one can understand how it was that they subsequently produced equine pernicious Anaemia by blood inoculation. Faulty technique while carrying out experiments with a number of horses may also be responsible for certain cases of pernicious anaemia. This was the experience here, when on one or two occasions pernicious anaemia was transmitted from virus reservoirs during the inoculation of horse-sickness virus or anti-horse-sickness serum, without our knowledge at the time. In case of one experiment, where this was proved to be the case, the mortality from pernicious anaemia was about 60 per cent. By subsequent transfusion experiments made from the surviving horses it was shown that two horses of the batch injected were the so-called "virus reservoirs," and responsible for the damage. The infection from these must have been carried to the healthy horses by the instruments.

5. Under the heading "Therapeutic Investigations," Seyderhelm and Seyderhelm have failed to prove that they were successful to effect recovery by their treatment (removing the *Gastrophilus* larvae from the stomach of the horse), if we remember, as pointed out above, that a number of horses recover, clinically, without any treatment. They do not give full particulars how they prepare their anti-pernicious anaemia serum. They only state that healthy horses are immunized with sublethal doses of the extracts from *Gastrophilus* larvae. As regards the good results obtained, e.g. by Beckmann in curing nine horses out of sixteen, the above remarks also apply, and, moreover, now that we have disproved the fact that *Gastrophilus* larvae is the cause of equine pernicious anaemia, we fail to realize how the above serum can be of any avail.

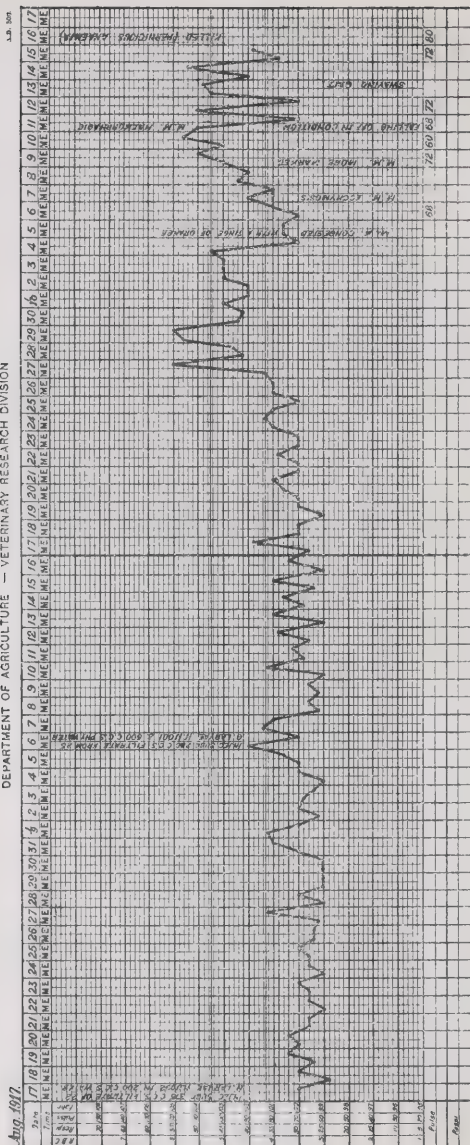
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DEPARTMENT OF AGRICULTURE — VETERINARY RESEARCH DIVISION

Aug. 1917.



A New Nematode in Fowls, having
a Termite as an Intermediary
Host.

[*Filaria Gallinarum* (nova species).]

BY

SIR ARNOLD THEILER, K.C.M.G.,
Director of Veterinary Research.

A New Nematode in Fowls, having a Termite as an Intermediary Host.*

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By Sir ARNOLD THEILER, K.C.M.G.,
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CIRCUMSTANCES LEADING TO THE DISCOVERY OF THE INTERMEDIARY HOST AND EXPERIMENTS TO FIND BOTH IMAGO AND ITS HOST.

Some years ago a farmer in the Orange Free State sent me a number of small nematodes, which he had obtained from a large white ant, called the "Houtkapper." He stated that by dropping the ants in hot water, it can be observed that the abdomen of some of the ants bursts, and the exit of the worm takes place. He at the same time asked the question, whether these worms were in any way connected with the wire-worms of sheep, with which his sheep were badly infected at the time. Such a connection had, of course, to be excluded, but that the worms represented a larval stage in the life-history of a nematode parasite in some animal was evident. Since termites are eaten by many birds, and especially probably by all insectivorous ones, the host had to be looked for amongst them. Fowls are in particular fond of termites. On many farms the custom exists of digging up nests of termites and allowing the chickens to feed on the exposed insects. A search for the houtkapper termite was then made at Onderstepoort and very soon infected individuals were found, very frequently in the neighbourhood of a Kaffir kraal, where fowls were running loose. Since kaffir fowls have to look for their food in the field, their droppings are spread all around the kraals and fields and serve as food for the termites. The termites according to Claude Fuller, to whom specimens were submitted, belong to *Hodotermes pretoriensis* (Fuller). The infected workers can easily be picked out amongst a number of non-infected ones; a distended abdomen, giving the insect a somewhat balloon-like appearance, is diagnostic. If such an abdomen is broken the larva wriggles out. It is most surprising to see a worm 20-30 mm. long come out of a termite's abdomen not more than 8.5 to 13 mm. in length and a width of 2.7 to 4.2 mm. The larval worm, as with such we have undoubtedly to do, is curled up in the coelomic cavity of the insect. It is of interest to note, that so far no soldiers were found to be infected with the larval nematode, a fact which would point to a different food being partaken of by them.

EXPERIMENTS TO REAR THE IMAGO BELONGING TO THE LARVA.

Since termites are eagerly eaten by fowls, the adult worm was expected to be found in these and most likely in their intestines. In order to exclude

* This paper was communicated to the Royal Society of South Africa on the 21st August, 1918.

mistakes, it was decided to feed clean chickens with infected termites, then to kill the birds after various periods and to search their intestines for nematodes, in which way definite results would be obtained. This experiment can be carried out with all prospect of success, provided care is taken to rear the necessary chickens free from parasites. This was done by hatching eggs in an incubator, keeping the chicks on clean ground and feeding them on clean food. These conditions were obtained by utilizing a pen with a cement floor containing sterilized sand as a run, and using Spratt's chicken food.

Experiment No. 1. Ten chickens reared in the manner described were placed in a cage and carted to a Kaffir kraal, where they were fed in the cage with termites freshly collected. This feeding was repeated on several occasions viz., in January, 1912, on the following dates:—11th, 16th, 17th, 19th, 23rd, 24th, 26th, and 29th; in February, on the 1st, 3rd, 8th, 13th, 16th, 20th, 24th, 27th, 28th, and 29th; and in March, on the 1st, 4th, 6th, 8th, 13th, 15th, 19th, 22nd. The results were as follows:—

(1) On the 18th March, 1912, one of the birds (No. 9) was found dead. On post-mortem examination numerous adult nematodes, males and females, all of the same species, were found in the jejunum.

(2) and (3). On the 19th March, 1912, birds Nos. 1 and 3 were killed. One adult nematode was found in the stomach of the first one and numerous nematodes in the jejunum of both.

(4) and (5). On the 20th March, 1912, birds Nos. 8 and 16 were killed. Numerous adults were found in the stomach and jejunum of the former and in the jejunum of the latter.

(6) On the 22nd March, 1912, bird No. 2 was killed and numerous nematodes were found in the jejunum.

(7) On the 12th May, 1912, bird No. 6 died. In the jejunum six adult worms were found.

(8) On the 22nd May, 1912, bird No. 7 died. In the jejunum two nematodes were found.

(9) and (10). On the 28th May, 1912, birds Nos. 14 and 15 were killed. The former one had been in bad health for some weeks. It had been kept isolated in a cage. It was found badly infected with mites. No nematodes were found in the intestines. Mites were also present in the second bird, which showed five nematodes in the jejunum.

Summary of Results.—Out of ten birds fed on termites infected with larval nematodes nine developed adult nematodes, all belonging to the same species.

Control to Experiment No. 1.—A number of fowls were selected to act as controls and were killed at various intervals in the course of the above experiment. The chickens belonged to the same incubator set as those used above, and had been reared in the same way. In addition a number of older fowls were killed that had been running free in the yard and had picked their food at random. The following results were obtained:—

(1) Three chicks, ten weeks old, were killed on the 20th March, 1912. No nematodes were found on post-mortem.

(2) An old cock and two hens were killed on the 22nd March, 1912. All three birds showed a species of tapeworm and in the caecum nematodes, but not of the species under consideration (*Heterakis*).

(3) A three-, a four-, and a six-months-old chicken were killed on the 27th March, 1912. All three were found free from nematodes.

(4) Two six-months-old chickens, that had been running free, were killed on the 26th March, 1912. Both were found free from nematodes.

(5) An old free-running hen was killed on the 4th April, 1912. A species of tapeworm and nematodes were found in the caecum, but not the one under consideration.

(6) A chick of the incubator set was killed on the 13th May, 1912, and found free from nematodes.

(7) Another chick of the same lot was killed on the 28th May, 1912, and found free from nematodes.

Summary of Results.—Of fourteen controls not fed with infected termites, none were found to contain the specific adult nematode. In the case of the specially-cared-for chickens no nematodes at all were found. In the case of the four free-running old hens, nematodes of a different species were present (*Heterakis*). This result was expected, the species of termites not being present on the ground on which the old hens were running.

Experiment No. 2.—This was carried out with three sets of chickens, all of the same brood, that had been reared and fed alike. One set of three chicks was to receive the larvae liberated from the termites, a second set of three the infected termites, and a set of six to serve as controls. For the purpose of the experiment the chickens were kept separately in a cage, the floor of which was removed every day and disinfected. The necessary grit was added to the food after sterilization. Chickens Nos. 26, 27, and 28 were fed with the living worms freshly collected and after having been rinsed in physiological water. During the month of April, on twenty-one different occasions each fowl obtained a daily average of about twenty worms, and during May, on seventeen occasions an average of about ten larvae. During the same period and on the same dates, chickens Nos. 29, 30, and 31 received infected termites.

Results.—On the 18th April, 1912, a chicken belonging to each lot and two controls were killed. Adult nematodes were present in the two former ones, but none in the controls. On the 15th May, 1912, and 4th June, 1912, two more controls on each date were killed and found free from nematodes. On the 17th June, 1912, the rest of the birds were killed. Adult nematodes were found in the chickens fed with larvae and termites, but none in the controls.

Conclusion. In these experiments positive results were again obtained. The larvae fed free or in termites developed to adults, all belonging to the same species. It is possible that in the negative case of experiment No. 1 the infection had died spontaneously, it having been observed in some occasions that adult worms were found in the faeces.

DESCRIPTION AND IDENTIFICATION OF THE NEMATODE.

(*Filaria gallinarum*, nova species.)

Origin of Material.—The adults were collected in all cases from artificially infected fowls. They were removed from the intestines soon after death and placed in a mixture of 70 per cent. alcohol, 10 per cent. glycerine, heated to near boiling point as prescribed by Looss and preserved in this liquid. This was the best method of rendering the opaque worms somewhat translucent, but a

complete clearing was not possible. The worms were not suitable for this purpose, the muscular sheath proving too thick, hence the inner structure was somewhat difficult to discern. It could, however, be ascertained after cutting some of the worms into pieces and tearing these asunder. Cross-sections were also made and proved useful.

Imago: Length.—The filiform body of the adults in male and female was of different length. This varied in the female between 60 mm. and 110 mm. The average length calculated in the case of twenty females amounted to 80 mm.; five individuals possessed this length, six were longer and nine shorter, which varied from 60 mm. to 69 mm. The average length of the males out of nine measurements was calculated to be 33 mm.; it varied between 28 to 40 mm. The average calculated length was found in one instance, four being above and four being below this average.

Shape and thickness.—The body was cylindrical filiform and attenuated at both ends; both head and tail were well marked. The diameter of the body at the nervous commissure varied from 352μ to 400μ , the distance from here to the oral end being of the same length. The greatest thickness was found to be at the end of the oesophagus or shortly behind it, at a distance, in three cases measured, from 3520μ to 4000μ , behind the oral end; the corresponding thickness measured from 736μ to 784μ . This thickness was maintained throughout the greater length of the body, which tapers gradually out when approaching the tail end, at the end of the chyle intestines it measured from 400μ to 480μ . The distance from the anus to the tip of the tail measured 880μ to 960μ ; the thickness at the level of the anus 320μ to 368μ .

Male.—The male was thinner than the female. The thickness at the nervous commissure varied from 288μ to 369μ , and the distance from here to the oral end 320μ to 368μ . The greatest thickness was also found at the level of the oesophageal end, at a distance of 3520μ behind the mouth, measuring 672μ to 688μ . The thickness at the level of the end of the chyle intestines varied from 320μ to 368μ ; the distance from anus to tip of tail 352μ to 400μ ; the thickness at the level of the anus from 192μ to 240μ . The distance from anus to tail-end was about half that found in females.

Cuticle.—The cuticle was ringed. The margin of the body in optical cross-section appeared indented (serrated), the posterior edges being in particular sharply marked and slightly projecting over the succeeding ones. The length of the rings measured from 8μ to 10μ . The thickness of the cuticle near the posterior extremity averaged from 32μ to 36μ . On cross-section it appeared that the cuticle consisted of five different strata varying in thickness, the thinner ones being situated towards the periphery. In a section taken from the middle of the body the following measurements were obtained: outer stratum 4μ thick, the second stratum 3μ thick, the third stratum 4μ thick, the fourth stratum 15μ thick, and the fifth stratum 10μ thick in all 36μ thick. Below the cuticle of the tail-end, laterally placed, was a small group of wartlike protuberances.

Subcuticle. The subcuticle was about 4μ thick and of a fine granular appearance.

Longitudinal bands.—The lateral longitudinal bands were distinct under the cuticle and of a fine granular structure. They were found to be about 3μ broad. On cross-section it could be noticed, that the lateral band was formed by the subcuticular substance, penetrating in the middle line between two muscular quadrants and bulging into the coelomic cavity to a depth of 200μ . At its passage through the muscular sheath it formed a narrow strip 20μ in width; it widened out inside to a diameter of 200μ so that the band in

the coelomic cavity represented on section an almost quadrangular outline. Near its inner surface it possessed a canal of semilunar shape. The dorsal and ventral longitudinal bands could not be recognized from the outside. On cross-section they appeared as a narrow strip of subcuticular substance, which just reached the inner surface of the muscular sheath.

The head was set off by a slight constriction and consisted of two lips, laterally placed, and separated by a slit; in the female the diameter of the head was 144μ to 168μ , and the length 64μ to 80μ . The outer surface of the head—that is, practically the outer surface of the lips—was smooth. The frontal side had a semicircular appearance and was obtuse, flat; the ring of the cuticle was present on the lateral side, but stopped short on the edge of the obtuse frontal side. The cuticle of the head was 8μ thick. Behind the constriction it formed a shoulder measuring 24μ to 32μ in thickness. The inner surfaces of the lips were sub-divided by two ridges in three cuplike grooves, lined by a thick cuticle (distinctly seen in lateral view). On the frontal side of the lip, just in front of the first ring, were two papillae, one placed dorsally and the other ventrally, that penetrated the cuticle in the shape of a thin thornlike process. Between these two, but situated behind, was seen a third papilla of a blunt knoblike process that did not penetrate the cuticle, which here formed a flat dent. Behind it at a short distance was the thornlike cervical papilla standing on a circular footplate.

The mouth. The slitlike opening between the two lips tapered down and entered into a short cylindrical tube, which rested on the oral end of the oesophagus, the entry into which was tri-radiate. The tube was lined with chitin.

Oesophagus.—This was a cylindrical tube 3520μ to 4000μ long externally discernible with some difficulty from the chyle intestines. The outer wall of the posterior end was but slightly constricted and ran continuous with the outer surface of the chyle intestine; the inner wall seemed to invaginate into the lumen of the intestine forming a short cylindrical projecting tube. The diameter of the oesophagus in a male at the constriction was found to be 160μ ; 100μ in front of this constriction the diameter was 208μ ; below the nerve commissure the diameter was 96μ . On cross-section the oesophagus was circular. The lumen was tri-radiate, dividing the wall into three sectors. In the oesophagus of a female at a place 352μ in diameter, the thickness of a sector measured 160μ . In the centre of each sector was found a lentiform layer of granular substance (oesophageal gland). The lumen was lined with a homogeneous membrane as well as its surface. It measured 2μ in thickness. The cross-section of the oesophagus showed a radiate striation (muscles).

Musculature. The musculature was well developed throughout the length of the body. Viewed laterally a system of muscular fibres could be seen arranged in groups succeeding each other along the body. Viewed on cross-section a muscular group consisted of four or five cells differing in length. Each cell consisted of a protoplasmatic portion bulging out inwardly, and a stem formed by the two striated walls, close to each other, ending below the subcuticle. The largest cell stood in the middle of the group, and on both sides joined to it were the three smaller ones arranged in descending order of length; at the stem of the large middle cell frequently a small one was fitted in. The protoplasmatic portions of the larger cells bulging inward touched each other, and the inner wall of the musculature was formed by these protoplasmatic portions. The number of the cells seemed to remain constant at thirty-two in a quadrant. The thickness of a striated muscle wall measured 4μ , that of the protoplasmatic portion 24μ to 30μ . The thickness of the muscular sheath was found to amount to 88μ .

Chyle intestine. It ran in a straight direction to the rectum, viz., without forming any loops or deviation, having everywhere the same diameter, viz. 416μ . It had a fine granular appearance, but no details could be recognized when examining through the body wall. The cross-section of the intestine appeared rather flat, semicircular, or even semilunar; with the longest diameter of 200μ . The walls were 24μ in thickness, a homogeneous inside membrane was 3μ thick, and the outside lining 4μ . The length of the rectum in a female was 480μ . The wall appeared finely striated radially, the striae being alternatively of homogeneous and granular structure. The posterior portion of the chyle intestine narrowed suddenly down to the rectum, which ran for a distance of 320μ in a dorso-ventral direction. Around the end portion of the chyle intestine ran a circular band 24μ broad, the recto-intestinal ligament. Above the rectum was spread out a fan-shaped muscle, 272μ broad at its insertion into the rectum, and 640μ on its insertion on the subcuticle of the back. The anus in the female possessed laterally a small wing.

Female sexual apparatus. In the frontal portion at a distance of 16 mm. to 20 mm. of the body was the vagina, situated ventrally, with a diameter of 80μ to 96μ , a simple circular opening without any appendages or thickening of the cuticle, leading through the cuticle and the muscular sheath as a simple cylindrical tube into the coelomic cavity. On the inner surface of the muscular sheath it turned almost at right angles backwards into a duct of 80μ to 120μ in thickness, lined with large polyedric cells. This duct formed a loop, with a small appendix in the convex curvature of the loop, and then split in a sharp angle into two branches, both running parallel, or the two branches went off almost in opposite directions, one, however, turning back sharply and running parallel with its mate. The branches widened out, they formed the uterus and contained numerous eggs; the eggs measured 40μ by 24μ . They suddenly narrowed down to the ovary, a thin long tube consisting of cells, the ovogonia being closely packed around the rachis.

The male sexual apparatus.—The tail end of a male was slightly curved ventrally. The cuticle of this portion was not smooth, but was covered with oval or rectangular shaped and linearly placed tubercles or shields; it formed on either side of the body a winglike ridge, containing six papillae. This wing started 148μ in front of the anterior papilla, its anterior border was rectangular to the axis, and between the wings was situated the anus, the anterior side of which was slightly raised to form a protuberance. Four of the papillae were placed in front of the anus and two behind. They were of the same length, 96μ , and were club-shaped, the club traversing the cuticle on the edge of the ridge through a circular plate. There were two spicules of unequal length, the smaller one was 200μ in length and was situated on the right side, the larger one was 800μ long and was placed on the left side. The longer spiculum ended thinly, the smaller one bluntly and surrounded by some kind of a sheath. The seminal duct formed in its course a convolute, it gradually thinned out, forming a loop at the junction of the oesophagus and chyle intestine, turning backwards and ending in the last third of the body length. The vas deferens ran ventrally parallel to the chyle intestine and entered into the funnel-shaped cloaca. The tail-end was slightly curved, the end of the body was twisted to form a spiral.

Nervous system.—The commissure surrounded the oesophagus 320μ behind the oral extremity and was 40μ thick. Laterally and projecting before and behind the commissure were placed the ganglion cells. A group of ganglion cells was also found under the fanlike muscle; above the rectum and dorsally from the anus were three fairly large cells, nuclei with nucleoli, were easily discernible in these cells.

Conclusion. The description given here corresponds best with that of a filaria, and since the species is a new one, as far as I can gather from the literature at my disposal, I propose to call it *Filaria gallinarum*.

Larvae in termites.—In the examination of the larvae similar difficulties were met with as in the adult, but by reason of their smaller size these difficulties were overcome to a large extent. The head was set off as in the imago. The two semi-circular lips were constricted at their base and separated from each other by a slit, the mouth opening. This opening led into a short cylindrical tube (the mouth cavity) lined with a homogeneous membrane, a continuation of the lining of the lips. The floor was formed by the oesophagus, the tri-radiate opening being recognized. The proximal portion of the oesophagus was slightly thicker than the succeeding one. The colour of the smaller larvae was white, that of the larger ones yellowish-brown. At the frontal side of the lips were the papillae arranged as found in the imago. The shape was a copy of the adult worms, with smaller dimensions. The length varied from 12 mm. to 35 mm. In a larva of 14 mm. length the thickest portion was that at or immediately behind the end of the oesophagus; it measured 352μ at a distance of 2160μ behind the oral end. The thickness at the level of the nervous commissure was 168μ at a distance of 224μ from the oral end. The width of the head (lip) was 128μ . In a larva of 18 mm. length the measurements were as follows: distance oral end to end of oesophagus 2720μ with a corresponding thickness of 528μ ; distance oral end to nervous commissure 256μ with corresponding thickness at this place of 288μ ; length of the head (lip) 64μ and width 128μ ; thickness at the level of the intestinal end 288μ ; distance anus to tip of tail 272μ , and thickness of 144μ at the anus. In a 32 mm. larva the measurements were as follows: distance oral end to oesophageal end 3200μ ; thickness at the level of this end 312μ ; distance oral end to nervous ring 304μ and corresponding thickness 320μ ; thickness of the lips 160μ ; thickness at intestinal end 320μ ; distance anus to tip of tail 480μ .

Cuticle.—The cuticle was ringed, the posterior margin of the ring was sharp, projecting toothlike when viewed sideways. The cuticle behind the constriction at the base of the lips stood out like a shoulder. In the tail laterally between anus and tip of tail a round disc was seen (a papilla) towards which the nearest rings were converging. Occasionally a ring was split into two rings in one half of the circumference, the ring in the middle line bifurcating into branches, both of which together were thicker than the main stalk from which they sprung. These split rings were found to succeed at intervals, separated by a number of simple rings between them. There was, however, no regularity in the succession of these rings; the intervals were shorter at the head end than at the tail end.

Subcuticle.—The lateral band was distinct, its width as it approached the anterior extremity was more than doubled. It was widest just in front of the commissure, its sides converging as it entered into the lips. In its backward course it broadened out again as it approached the posterior end of the chyle intestine, and was very broad at the junction of the rectum, tapering into the tail.

Musculature.—The same arrangement as in the adult was present in the smallest as well as in the largest larva.

Intestinal tract.—The oesophagus at its junction with the chyle intestine was slightly constricted and its inner walls projected into the lumen as described. The chyle intestine was straight. No details were discernible in its course. A ligament circled around its posterior end, which suddenly tapered into the rectum, forming a cone. On the surface of this cone, dorsally and

ventrally, a large oval nucleus with a nucleolus was recognized, probably belonging to the cells which formed the funnel-shaped rectum. The encircling ligament probably formed part of a sheath, surrounding the intestine, and was composed of two cells, a ventral and a dorsal one. In the former one a nucleus with nucleolus was particularly distinct. The lumen of the rectum possessed a homogeneous lining (chitin).

Nervous system.—Parts of it could be recognized and particularly so the commissure near the head. Around the commissure were grouped a number of ganglion cells. The lateral ganglion in particular was distinct, occupying the whole width of the lateral band. There were groups of cells situated ventrally and dorsally to the commissure, but not so well defined in outlines. There was a group of ganglion cells behind the anus in the triangle formed by the rectum and the ventral wall; the foremost cells were large and distinct; a second group was placed above the rectum and also in an oblique direction; also here the two foremost cells were large and distinct. Laterally, above the anus, was a third collection of large and distinct cells, with a finely granulated plasma.

NOTES ON THE EVOLUTION.

The mature eggs contained fully developed embryos. They possessed a thick shell, indicating that hatching is not likely to take place within the evacuated faeces. Such eggs, when put up for cultivation in flasks in the usual way, did not develop larvae. It must be assumed that the termites swallow the eggs and that the eggs hatch in their bodies. This hatching probably takes place somewhere in the intestinal tract and the larva finds its way into the coelomic cavity. So far, the larvae examined, with one single exception, whether short or long, were of identical structure. They must, therefore, belong to the same stage, this stage is the second larval stage. The one exception was the smallest larva found, it was in the process of ecdysis, distinctly surrounded by a skin; this is decidedly the first ecdysis. It must therefore be concluded that the larva moulting in the termite undergoes only one ecdysis in this intermediary host and reaches the definite host in its second stage and before it has undergone the second ecdysis. In the larvae found in the termites, no sexual primordium was seen.

Of a number of larvae (fifty) fed to a fowl during four succeeding days which was killed eight days after the feeding of the first or five days after the feeding of the last, twenty nematodes were recovered. All of these were still in the asexual form. This was characterized by a thick ring of the body. Below the old cuticle a new one could be recognized, the ring of which was much finer. It was present in all, but not equally distinct. In some, it was faintly indicated, and not even distinct in all parts of the body, whilst in others, it was well pronounced and distinct. In the most advanced ecdysis a fairly large space was noted between the cuticle of the tip of the tail and the body within and a distinct cuticular lining of the cloaca was present in these cases.

There could accordingly be no doubt that this stage represented the second ecdysis. The interesting part of this second ecdysis is thus the fact, that it does not take place in the intermediate host. In analogy to the free living stages of parasitic nematodes (*Haemonchus contortus*, *Trichostrongylus douglasii*), which conclude their second non-parasitic stage with an ecdysis, and are in their second skin capable of withstanding the most severe influences until they finally reach their host, a similar occurrence might be expected. In the filaria under discussion no free living larval stage is present, and this

fact excludes the necessity of provision being made for special protection or for longevity. On the contrary it would appear to be in the interest of the species, that the second larval stage only undergoes its second ecdysis in the final host, thus increasing the chances of reaching the final evolution. Since in the evolution of nematodes four different ecdyses are observed we expect to find two more stages in the evolution of the filaria under discussion. What may be considered the third stage was found amongst nematodes collected from a fowl that had been fed during a few days with a number of infected termites and was killed three weeks after the first termites had been supplied. A male nematode could distinctly be recognized by the presence of the sexual tube, that started about in the end third of the body as a loop, the end turned backwards, forming two convolutions, then in a straight line it ran below the intestinal tract into the cloaca. This nematode possessed only one cuticle, the ringing of which was very fine. It must thus be surmised that it was at the beginning of the third stage. This view is supported by the observation that in the tail end the male characters were present. (Corresponding to this male stage was found a female stage. The sexual tubes were short, the vagina opened on to the surface of the cuticle which also was finely ringed, somewhat coarser than that of the male, but finer than that of the second larval stage. Both ends of the uterus were curved in the opposite direction a good distance behind and in front of the oesophageal and caudal end, thus showing their comparatively young age. Amongst a number of nematodes found in a fowl killed seventeen and eighteen days after feeding the larvae, female nematodes were found in the process of moulting, the outer skin being only separated at the tail end. The sexual tubes within were thin and not fully developed. They were probably at the beginning of the third ecdysis. Quite a number of worms were found in the process of ecdysis, what may be interpreted as the fourth stage. In the male the germinal tube was quite distinct and filled about half to two-thirds of the ventral length. At the tail-end the six lateral papillae were present and the flap with its cuticular shields. In some worms was seen what may be described the "anlage" of the spicules. There were two cuticules present. The old one was detached on the tail-end, and it was here that the loosening first began, apparently to make room for the two caudal flaps. Again, the ringing of the inner cuticle was finer than that of the outer. The females of the same stage were also found undergoing ecdysis. The vagina did not penetrate the outer cuticle and was not completely differentiated, the sexual tubes were not fully developed. Again outer and inner cuticules could be recognized, the inner one finely ringed. The inner one was not distinct in all worms seen. A separation of the two could in some instances be seen at the tail-end. Compared with the females found undergoing the third ecdysis these were slightly further advanced in their sexual organs, but no other differences could be made out. The presence of four stages in the evolution of this worm could thus be made out, although as far as the end of the third and the beginning of the fourth was concerned specimens were wanting. The observations made are, therefore, also open to a different interpretation. It seems to be beyond doubt that the second ecdysis takes place in the fowl. A stage has been seen, that was interpreted as the beginning of the third one and one as the third ecdysis in the case of females, but the greater majority of worms of the same fowl were considered to represent the fourth ecdysis. Since in the male nematodes the differentiation of the tail-end within the skin corresponded entirely with that of the imago, the interpretation as the fourth ecdysis is justified. No male specimens were found that could be interpreted to represent the third ecdysis and none of the beginning of the fourth stage. Hence what has been interpreted as the fourth ecdysis may in fact be the ecdysis of the third stage. The same applies

to the female stage. What is considered to be the third and fourth ecdysis may only be an earlier and later stage of one and the same, viz., the third ecdysis. Thus in this nematode instead of the usual four stages of the free-living and half-parasitic nematodes only three may be present, one being suppressed. Such a suppression could be explained, if it is considered that the skin of the second ecdysis in the half-parasitic nematodes (*Haemonchus*, *Trichostrongylus*, *Ankylostomum*) is meant to act as a protection for the third stage until it had reached its host, such a protection in the case of an intermediary host not being necessary. In support of this view would be the fact, that mature adults were found ten days after feeding of the larvae, as was the case in fowl No. 107. This bird received on the 14th and 16th March a number of larvae. On the 24th March, viz., ten days after the first and eight days after the second feeding it was killed and twenty-nine nematodes were collected. Of these fifteen were males, twelve females, and two asexual larvae. The males were undergoing ecdysis and differentiation of the typical tail-end had taken place in most of them, all females were likewise undergoing the process of ecdysis. If three ecdyses were required to take place in the bird, there would indeed be but a very limited time for it, at the utmost ten days. In *Trichostrongylus douglasii*, the third moulting takes place at an average of four days after the entry of the mature larvae and the fourth stage fifteen days later or about the twenty-first day after entry. In *Haemonchus contortus* the fourth ecdysis is noted between the ninth and eleventh day, the third ecdysis is reached at the conclusion of forty-eight hours after the intake of the mature larvae, the fourth stage requiring again the longest period. The suggestion that what corresponds to the third stage in the half-parasitic nematode is suppressed in the filaria under discussion is, therefore, quite a feasible one. Whilst in this one fowl the evolution of the nematodes had taken place within a comparatively short period, this did not seem to be the rule. In the fowls Nos. 104 and 105 that had been killed eight days after the first or five days after the last feeding of larvae only asexual larvae in the process of ecdysis were found. In fowl No. 101 that was killed two days after feeding of larvae, and in fowls that were killed four, three, two, and one day respectively after feeding of larvae the nematodes with two cuticules were present, in some the inner ring was faintly present, in others quite distinct; in fowl No. 102, killed five and three days respectively after feeding, the same was noticed. It is possible that the different larvae require different lengths of time for their evolution. This would find an explanation in view of the fact that in the termites not all larvae are in the same stage when eaten by the fowl—those most advanced would require a shorter period than those that are as yet young. Fully developed adult males and females were found in a fowl that was killed three weeks after its first feeding. The experiments have thus not definitely settled whether, in the evolution of the filaria under discussion, three or four larval stages are present. The evidence at disposal can be interpreted in favour of both. It is, however, most likely that the general rule is still kept and that the interpretation of four ecdyses is the correct one.

EXPLANATION OF PLATES.

(A New Nematode in Fowls.)

Fig. 1.—*Hodothermes pretoriensis* (Fuller).

Filaria gallinarum (spec. nov.).

Two workers with larvae in the abdomen.

One shows the larva escaping from its body after it had been placed in hot water. The size of a larva is shown at the right side. Male and female nematodes are shown below.



FIG. 1.

Anoplura from South African Hosts.

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Anoplura from South African Hosts.

By G. A. H. BEDFORD, F.E.S., Entomologist, Division of Veterinary Research, Onderstepoort.

THE present paper is based upon the material in the laboratory collection, of which the majority has been collected in the Transvaal by Mr. Powell and myself. The collection also contains a number of species which have been collected in Natal by Mr. Hill, the Veterinary Research Laboratory, Maritzburg, and a few species have been very kindly presented by Mr. E. C. Chubb, Curator of the Durban Museum. To Dr. Breyer I am indebted for kindly placing the whole of the collection of Anoplura (which was collected by Mr. C. J. Swierstra) in the Transvaal Museum at my disposal, and for kindly allowing me to examine skins in the museum for parasites. The Anoplura listed include the majority of the species which are likely to be found on domestic animals and birds. Unfortunately I have only been able to enumerate a small percentage of the species in the collection owing to my not having had access to all the literature on the group and as I have not had time to work up a large quantity of material, which has only recently come to hand. No attempt has been made here to include all the previous records of species which have been found on South African hosts, because our knowledge at present is very limited and any such list would be far from complete, but it is the author's intention to give a host-list of all the known species found on South African mammals and birds at a future date, when the whole collection has been worked up and more material added.

In this paper the recent classification of Harrison * has been followed.

No attempt has been made to give a complete synonymy of all the species listed, and as a rule only well-known specific names, which have recently been sunk as synonyms, have been included.

* Harrison, L. : "The Genera and Species of Mallophaga." Parasitology, Vol. 9, No. 1, 1916

ORDER ANOPLURA.

Sub-order Siphunculata, Meinert.

FAMILY PEDICULIDAE.

Genus PEDICULUS, Linné.

Pediculus, Linné, *Syst. Nat.*, p. 610 (1758).

1. *Pediculus humanus*, Linné (1758).

- | | | |
|--|---|-------------------------|
| Syns. <i>P. capitis</i> , De Geer (1778)
<i>P. cervicalis</i> , Latreille (1803)
<i>P. consobrinus</i> , Piaget (1880) | } | Lice found on the head. |
| <i>P. corporis</i> , De Geer (1778)
<i>P. vestimenti</i> , Nitzsch (1818)
<i>P. tabescentium</i> , Alt (1824) | } | Lice found on the body. |

This species is very common on man, especially among the native races in South Africa, and Mr. Brain, of the Entomological Division, informs me that they are more numerous amongst the natives in Queenstown than in any other town in the Union.

The lice found on the body have, until recently, been regarded as specifically distinct from those found on the head. The chief differences between them are that the body lice are usually larger, with more hairs on the integument, the thorax broader in proportion to its length, and the lateral borders of the abdomen less festooned, but all these characters vary in different specimens. Especially is this the case with specimens found on different races of man, and Patton and Cragg*, who have studied this louse in India, state that in many instances they have found it impossible to decide whether a given specimen should be regarded as *capitis* or *vestimenti*.

The head lice found on Europeans are much lighter in colour than those found on darker races, and are more common in South Africa than the forms found on the bodies of their hosts.

Genus PHIRUS, Leach.

Phirus, Leach, in Brewster. *Edinburgh Encycl.*, Vol. ix, p. 77 (1815).
Phirius, Burmeister, *Handb. Ent.*, Vol. ii, p. 52 (1835).

1. *Phirus pubis*, Linné (1758).

Syn. *P. inguinalis*, Leach (1815).

This species is also found on man throughout the Union, but is not so common as the foregoing species. As its name implies, it is usually found about the pubic region.

* Patton and Cragg: *Medical Entomology*, p. 546, 1913.

Genus *PEDICINUS*, Gervais.

Pedicinus, Gervais, Hist. Nat. Ins. Apteres, Vol iii, p. 301 (1847).

1. *Pedicinus* sp.

Several females from a Vervet (*Cercopithecus pygerythrus*), Fairfield, Rustenburg District, Transvaal (W. Powell).

2. *Pedicinus* sp.

Several females from a Chacma Baboon (*Papio porcarius*), Mooi Vlei, Transvaal, 28th July, 1916 (W. Powell).

FAMILY HAEMATOPINIDAE.

Genus *HAEMATOPINUS*, Leach.

Haematopinus, Leach, Zool. Miscell., Vol iii, pp. 64 and 65 (1817).

1. *Haematopinus asini*, Linné (1758).

Syn. *Haematopinus macrocephalus* Burm. (1838).

Common on horses and donkeys throughout the Union.

Fernandes * has recorded it occurring on horses and mules in Mozambique.

It has also been taken from a Burchell Zebra (*Equus burchelli*).

2. *Haematopinus eurysternus*, Nitzsch (1818).

Common on cattle in South Africa. Howard † has recorded it being common on cattle throughout Mozambique.

3. *Haematopinus suis*, Linné (1758).

Syn. *Haematopinus urius*, Nitzsch (1818).

This species is often common on pigs at Onderstepoort. Numerous specimens have also been received from Mr. A. L. Hill, the Veterinary Research Laboratory, Maritzburg, Natal.

Howard has also recorded it having been taken off pigs at Umbelusi.

4. *Haematopinus phachochoeri*, Enderlein (1908).

Syns. *Haematopinus latus*, Neumann (1908).

Haematopinus peristictus, Kellogg & Paine (1911).

Haematopinus phachochoeri, Paine (1912).

Haematopinus incisus, Harms (1912).

Haematopinus phachochoeri, Harms (1912).

One female from a Warthog (*Phacochoerus aethiopicus*), Rustenburg District, Transvaal; also a number of females and males from the same host, Bridgewater, Rustenburg District (W. Powell).

* Fernandes, J.: "Phthiriasis." Boletim de Repartição de Agric., Lorenzo Marques, 1914.

† Howard, C. W.: "Insects directly or indirectly injurious to man and animals in Mozambique." Bull. of Ento. Res., Vol. iii, Part ii.

Ferris has recorded this species from *Potamochoerus chaeropotamus*, Ngxwala Hill, Ubombo, Zululana.

It has also been recorded from other parts of Africa from the following hosts:

Phacochoerus aelianus massaicus, *P. aethiopicus*, *Potamochoerus choeropotamus*, and *P. africanus*.

No doubt this species will eventually be found to be common on warthogs and bushpigs throughout the Ethiopian region.

Genus *LINOGNATHUS*, Enderlein.

Trichaulus, Enderlein, Zool. Anzeig., Vol. xxviii, pp. 139, 141 (1904).

Linognathus, Enderlein, Zool. Anzeig., Vol. xxix, p. 194 (1905).

1. *Linognathus vituli*, Linné (1758).

This species is common on cattle, especially calves, in the Transvaal. Fernandes has recorded it from Mozambique.

2. *Linognathus setosus*, Olfers (1816).

Syn. *L. piliferus*, Burmeister (1838).

This louse does not appear to be at all common in the Transvaal. I have only taken one female off a dog at Onderstepoort. Waterston has recorded it having been taken off a dog at Capetown, and it has been recorded from Mozambique by Fernandes.

3. *Linognathus* sp.

Three females taken from a sheep in Natal (A. W. Shilston). There are altogether three species having been found on sheep, namely, *L. africanus*, Kellogg & Paine (*L. stenopsis*, Burm.) taken on sheep in Nigeria; *L. pedalis*, Osborn, found on sheep in North America and Australia; and *L. ovillus*, Neumann, which was described from specimens taken off sheep in Australia.

The specimens in the collection may be *L. ovillus*, Neumann, but as I have not seen Neumann's description I am unable to state whether it is his species or whether it is a new one.

They are closely allied to *L. setosus*, Olfers.

4. *Linognathus stenopsis*, Burmeister (1838).

This species is common on goats in the Transvaal and Natal. Fernandes has recorded it from Mozambique.

5. *Linognathus fahrenheitzi*, Paine (1914).

Syn. *L. forficula*, Kellogg and Paine (1911).

Four specimens, all females, taken on a Mountain Reedbuck (*Cervicapra fulvorufula*) at Mfongosi, Zululand (collected by Mr. Jones and presented by Mr. Chubb, Curator of the Durban Museum).

6. *Linognathus unguolata*, Piaget (1885).

Two females from a Red Duiker (*Cephalopus natalensis*), Mfongosi, Zululand (collected by Mr. Jones and presented by Mr. Chubb).

7. *Linognathus caviae-capensis*, Pallas (1767).

Several females from a Cape Hyrax (*Procavia capensis*), Rooi Krans, Rustenburg District, Transvaal (W. Powell).

Genus *SCIPIO*, Cummings.

Scipio, Cummings, Bull. Ento. Res., Vol. iii, p. 393 (1913).

1. *Scipio aulacodi*, Neumann (1911).

Several females taken from a Cane-Rat (*Thryonomys aulacodus*) in the Rustenburg District, Transvaal (W. Powell).

This species has been found on the same host in Dahomey and North-East Rhodesia; also from a *Thryonomys* sp. at Mfongosi, Zululand.

2. *Scipio breviceps*, Ferris (1916).

Four females taken from the same individual as the preceding species.

This species was described from specimens taken off a *Thryonomys* sp. at Mfongosi, Zululand.

Genus *HYBOPHTHIRUS*, Enderlein.

Hybophthirus, Enderlein, Denksch. der Medig.-Naturw. Gesells (1909).

1. *Hybophthirus notophallus*, Neumann (1909).

Numerous specimens, mostly females, taken from an Ant-bear (*Orycteropus capensis*) in the Zoo, Pretoria, 24th March, 1914 (C. J. Swierstra).

Genus *POLYPLAX*, Enderlein.

Polyplax, Enderlein, Zool. Anzeig., Vol. xxviii, pp. 139, 142, and 223 (1904).

1. *Polyplax spinulosa*, Burmeister (1839).

A number of females, males, and immature forms of this species have been taken from two or three species of rats at Onderstepoort. The collection also contains several ♀♀, ♂♂, and immature specimens taken off *Mus decumanus* at Maritzburg, Natal (A. L. Hill).

2. *Polyplax otomydis*, Cummings (1912).

This species is extremely common on the Water-rat (*Otomys irroratus*) at Onderstepoort. The collection also contains specimens taken off the same host at Jericho, Transvaal, and Mfongosi, Zululand.

Waterston has recorded it from *Otomys brantsi luteolus*, from skins in the South African Museum, Capetown.

It was described by Cummings taken from *Otomys irroratus tropicus* in British East Africa.

3. *Polyplax waterstoni*, nov. sp. (Plate I, figs. 1, 2, 4, and 5.)

A number of males and females from several rats (two species) at Onderstepoort.

Female: Length 1.46 mm., head .2 mm., thorax .13 mm., abdomen 1.13 mm., width of abdomen .48 mm.

Head: Almost as broad as long. Immediately behind the antennae the head widens abruptly and then becomes slightly narrower again. Behind the temporal margins the posterior margin turns

abruptly inwards and then becomes convex. One conspicuous hair on each side near the base of the antennae, and three backward projecting hairs at the posterior angles, the first of which is the shortest, the second about twice as long, and the third reaching to the thoracic stigma. On the ventral surface there is one longish hair on each side near the base of the antenna, and two short hairs on each side in a line with the anterior margins of the antennae.

Antennae: Five-jointed; the first joint slightly broader than long; the second nearly as long as the first, but much narrower, narrower at the base than at the apex; the third, fourth, and fifth joints small—about the same size. Sense-organ present on the fifth.

Thorax: Short, lateral margins convex, the anterior margin with a V-shaped notch, which extends as a narrow median furrow to the posterior border of the mesothorax. On the posterior margin of the mesothorax there are six hairs; the outer pair are short and situated near the lateral border; the second pair are twice as long as the first pair, and the third pair are long and extend to the abdomen. Sternal plate as in Plate 1, fig. 5.

Legs: The first pair are small with very small claws; second pair larger, claws also larger; third pair large with stout claws.

Abdomen: Elongate, broadest about the middle, rounded behind. On the dorsal surface segment i short with two hairs; segment ii with two rows of hairs, the first of two, the second of six; segment iii with one sclerite and one row of six hairs; segments iv to vii each with two sclerites and two rows of six to eight hairs. Segments viii and ix each with one sclerite and one row of six hairs. On the ventral surface segment i indistinct; segments ii to vii each with two sclerites and two rows of four to eight hairs; segment viii with one sclerite. Gonopods with three hairs, two long and one short.

Pleurae: Triangular with two long teeth on the posterior lateral margins and two short bristles; the teeth of the dorsal angle being the longer.

Pleurae of segment vii with a single tooth and two longish bristles; pleurae of segment viii without a tooth and two long bristles.

Male: Length 1.02 mm.; head .18 mm.; thorax .13 mm.; abdomen .71 mm.; width of abdomen .36 mm. Head, thorax, legs, and pleurae like those of the female. Third joint of antennae projected outwardly to a point. Posterior margin of mesothorax with only four hairs, two long and two short.

On the dorsal surface the abdominal segments have only one sclerite each. On the ventral surface segments ii and iii each have two sclerites, the remainder with one each. Distribution of hairs on the dorsal and ventral surfaces as in the female, except segment viii, which has four hairs on the dorsal surface and two median short spines on the venter.

Genitalia conspicuous. See Plate I, fig. 4.

I have much pleasure in naming the species after the Rev. James Waterston, B.D., B.Sc., of the Imperial Bureau of Entomology, to whom I am indebted for much kind assistance.

4. *P. arvicathus*, nov. sp.

Three females taken from the Striped Mouse (*Arvicanthus pumilio*) at Onderstepoort.

Female: Length 1.14 mm.; head .13 mm.; thorax .13 mm.; abdomen .78 mm.

Head: As broad as long; in shape it resembles that of *P. waterstoni*. On the dorsal surface in front of the antennae there is a transverse row of four hairs. Behind the antennae there is an inconspicuous transverse suture with six hairs. One minute hair at the margin of the temples and three backward projecting hairs near the posterior angles, the first and second short (about equal), the third long and extending to the thoracic stigma; also two minute hairs on the occiput. On the ventral surface four hairs in a line with the anterior margins of the antennae: two minute hairs immediately in front of these and two longer ones behind. Antennae five-jointed, the first joint broader than long; the second very nearly as long as the first, but narrower, about the same width at the base as at the apex; the third and fourth small, about the same size; the fifth short, with a sense organ.

Thorax: Short, lateral margins convex. The anterior margin concave. On the posterior margin of the mesothorax there are two long hairs which reach the abdomen: behind these there are two short hairs. Sternal plate as in Plate I, fig. 6.

Legs: First pair small with fairly long slender claws; second pair larger, claws also larger; third pair large with stout claws.

Abdomen: Elongate, broadest about the middle. On the dorsal surface: segment i short with two hairs; segment ii with two rows of hairs, the first of two the second of four; segment iii with one sclerite and eight hairs; segments iv to vii each with two sclerites and two rows of eight to ten hairs; segments viii and ix each with one sclerite and one row of six hairs on the former segment, and two median ones on the latter. On the ventral surface segment i indistinct; segments ii to vii each with two sclerites and two rows of four to ten hairs; segment viii with one sclerite. *Gonopods* with three hairs, one long and two short.

Pleurae as in Plate I, fig. 3.

Genus HOPLOPLEURA, Enderlein.

Hoplopleura, Enderlein, Zool. Anzeig., Vol. xxviii, p. 221 (1904).

1. *Hoplopleura intermedia*, Kellogg and Ferris (1915).

Numerous males and females from rats (two undetermined species) taken at Onderstepoort. This species was described from specimens taken on *Mus coucha* at Mfongosi, Zululand. The collection also contains several specimens, which Ferris * takes to be immature forms of this species.

Genus HAEMODIPSUS, Enderlein.

Haemodipsus, Enderlein, Zool. Anzeig., Vol. 28, pp. 139, 143 (1904).

1. *Haemodipsus ventricosus*, Denny (1842).

Several females, males, and immature forms collected from domestic rabbits at Onderstepoort.

* G. F. Ferris: "Mallophaga and Anoplura from South Africa." Annals of the Durban Museum, Vol. I, Part 3, pp. 243-245, fig. 27, 1916.

Sub-order Mallophaga.

FAMILY BOOPIDAE, Mjöberg.

Genus HETERODOXUS, Le Souef and Bullen.

Heterodoxus, Le Souef and Bullen, Vict. Naturalist, Vol. xviii, p. 159 (1902).

1. *Heterodoxus longitarsus*, Piaget (1880).

The collection contains numerous males and females taken from dogs at Onderstepoort, 22nd May, 1915 (G.A.H.B.); Mooi Vlei, Transvaal, 30th August, 1916 (W. Powell); and at Maritzburg, Natal (A. L. Hill).

It has also been found on dogs in other parts of Africa, America, Malay Peninsula, Japan, and Formosa, and is common on kangaroos and wallabies in Australia.

FAMILY GYROPIDAE, Burmeister.

Genus GYROPUS, Nitzsch.

Gyropus, Nitzsch, Germar's Magazine, iii, p. 303 (1818).

1. *Gyropus ovalis*, Nitzsch (1838).

This species is very common on Guinea-pigs (*Cavia cobaya*) at Onderstepoort.

Genus GLIRICOLA, Mjöberg.

Gliricola, Mjöberg, Arkiv. f. Zoologi, vi, p. 18 (1910).

1. *Gliricola porcelli*, Linné (1758).

Syn. *Gliricola gracilis*, Nitzsch (1838).

The collection contains numerous males and females taken off guinea-pigs at Onderstepoort.

FAMILY MENOPONIDAE, Mjöberg.

Genus MENOPON, Nitzsch.

Menopon, Nitzsch, Germar's Magazine, iii, p. 299 (1818).

1. *Menopon antennatum*, Kellogg and Paine (1911).

Several females and males taken off the Crowned Guinea-fowl (*Numida coronata*, Gray) at Bridgewater, Transvaal, 18th June, 1917, (W. Powell), also several females and males taken from a guinea-fowl at Maritzburg, Natal (A. L. Hill).

This species was described from specimens taken off *Numida mitrata* by H. H. King at Azzar, near Boe, Egyptian Sudan.

2. *Menopon madagascariense*, Mjöberg.

Several females and males collected from a Hammerhead (*Scopus umbetta*, Gmel.) in the Rustenburg District, Transvaal, and at Maritzburg, Natal (A. L. Hill). They differ from Mjöberg's description of his types, which were taken from the same host in Madagascar, by the presence of six hairs on the occipital margin of the head, and also by their having ten hairs instead of only six on the posterior margin of the prothorax. At each end of the transverse line there is a small spine.

3. *Menopon (Menacanthus) stamineum*, Nitzsch (1874).

Syn. *M. (M.) biseriatum*, Piaget (1880).

This species is extremely common on domestic fowls and turkeys at Onderstepoort, and on fowls at Maritzburg, Natal.

4. *Menopon (Menacanthus) numidae*, Giebel (1874).

The collection contains two ♀♀ taken off the Crowned Guinea-fowl (*Numida coronata*, Gray) at Elandsfontein, Transvaal, 11th April, 1916 (W. Powell). I have also taken it off domestic fowls at Onderstepoort on more than one occasion.

6. *Menopon (Menacanthus) giganteum*. Denny (1842).

Syn. *M. (M.) latum*, Piaget (1880).

One ♀ taken off a domestic pigeon at Onderstepoort, 2nd May, 1914 (G.A.H.B.), and one ♀ from a Cape Turtle-dove (*Turtur capicola*, Sund.), Brock Spruit, Transvaal, 2nd April, 1916 (W. Powell).

7. *Menopon (Menacanthus) spinosum*, Piaget (1880).

One female collected from a Red-shouldered Glossy Starling (*Lamprocolius phoenicopterus*, Swains) at Jericho, Transvaal (W. Powell). Mr. Hill has also taken a female from a Blackcap Bulbul (*Pycnonotus barbatus layardi*) at Maritzburg.

Genus COLPOCEPHALUM, Nitzsch.

Colpocephalum, Nitzsch, Germar's Magazine, iii, p. 298 (1818).

1. *Colpocephalum turbinatum*, Denny (1842).

Syn. *C. longicaudatum*, Nitzsch (1866).

Several females and males taken off a domestic pigeon at Maritzburg (A. L. Hill).

2. *Colpocephalum pygidiale*, Mjöberg (1910).

Several females and males of this distinct species taken from a Sacred Ibis (*Ibis religiosa*) in the Rustenburg District, Transvaal, 17th June, 1917 (W. Powell).

3. *Colpocephalum scopinum*, Mjöberg (1910).

Three ♀♀ and three ♂♂ taken from a Hammerhead (*Scopus umbretta*, Gmel.) at Maritzburg, Natal (A. L. Hill).

This species was described from specimens taken off the same host at Madagascar.

4. *Colpocephalum subpenicillatum*, Piaget (1885).

One ♂ from a Hadadab Ibis (*Hagedaschia hagedasch*, Lath) at Mfongosi, Zululand (collected by Mr. Jones and presented by Mr. Chubb).

5. *Colpocephalum harrisoni*, nov. sp.* (Plate II, fig. 7.)

Female: Length 3.226 mm.; width .85 mm.; ground-colour light yellowish brown. *Head*: Length .5 mm.; width .56 mm.; forehead conical, much narrower than hind head, with five short and one longish hair on each side of the median line, and five long hairs in front of the ocular emargination. Palpi long, last two segments projecting beyond the head. Antennae long, third joint with the basal half very narrow, and then gradually widening out towards the apex; fourth joint elongated-oval. Eye prominent, almost, if not quite, divided. The ocular emargination very pronounced; ocular fringe well developed; temples projecting, slightly angular in front, rounded behind, with six long hairs; occipital margin concave with four bristles; in front of the occipital margin there are two large black blotches which are connected together by a dark transverse band; ocular blotches also large and black. On the dorsal surface of the head there are two short admedian hairs in front of the blotches and three longer ones behind each blotch. Mandibles dark brown.

Prothorax: Short, narrower than the head; lateral angles produced with a short spine in each angle and two longer ones behind; just inside each lateral angle, at each end of the transverse line, there is a short hair; on the posterior margin there are four long lateral bristles and two short admedian hairs.

Mesothorax: Distinct, separated from the metathorax by an uncoloured band; slightly narrower in front than behind, with six short hairs on the posterior margin.

Metathorax: Wider than mesothorax, with several spines and hairs on the lateral margins.

Legs: Long and slender, with dark marginal bands on femora and tibiae; hind femur with a dense patch of small hairs on the ventral surface.

Abdomen: Very long and slender, broadest at segment iii. Segment i very short, about the same width as metathorax; segment ii considerably longer and broader. Tergites i to viii with a few hairs on the posterior margins and several short scattered hairs; tergite ix with three or four small hairs in the middle of the posterior margin, two long ones on the posterior angle and another long one just inside the angle. Segments iii and iv with the posterior margins concave. Ground-colour light yellowish brown, pleurae and lateral margins of the third and fourth segments slightly darker; ninth segment with two large square brown markings. Pleurae with several short spines. Pleurae of segments ii to ix, each with a single long hair and one or two short ones on the posterior margin. Sternites ii

* This species belongs to Ferris' new genus *Helconomus* (*Canadian Entomologist*, p. 305, September, 1916), which includes five species found on Cranes. The collection contains several species of *Malliplaga* from other Bustards, but there are no species of *Menop-nidae* amongst them, and as no species belonging to this family have previously been recorded from bustards, it is quite possible that the host from which the new species was taken was wrongly recorded, or the specimens may have been stragglers.

and iii with a patch of extremely minute hairs on each side of the middle line. Sternite iv with a dense patch of minute hairs crowded together on each side of the median line. Sternites ii to viii each with a number of fairly small, irregularly arranged hairs and a large, broad median band, which is concave on its posterior margin. Lip of the opening to the genital chamber with numerous fine hairs.

Described from two females taken off a Bustard (*Otis* sp.) in Angola (T. Meyer).

I should not have ventured to describe this species had it not been for Mr. Harrison, after whom I have had much pleasure in naming it.

Genus MYRSIDEA, Waterston.

Myrsidea, Waterston, Ent. Month. Mag., p. 12 (1915).

1. *Myrsidea rustica*, Nitzsch (1874).

One female taken from a House Martin (*Delichon urbica*) at Brock Spruit, Transvaal, 7th April, 1916 (W. Powell).

Genus TRINOTON, Nitzsch.

Trinoton, Nitzsch, Germar's Magazine, iii, p. 300 (1880).

1. *Trinoton anserinum*, Fabr. (1805).

Syns. *T. conspurcatum*, Nitzsch (1838).

T. continuum, Piaget (1880).

Several specimens, both females and males, taken from a Spur-Winged Goose (*Plectropterus gambensis*, Linné).

2. *Trinoton querquedulae*, Linné (1758).

Syns. *T. luridum*, Nitzsch (1838).

T. squalidum, Denny (1842).

Numerous specimens taken from the Red-billed Teal (*Anas erythrorhyncha*, Gm.), the South African Sheldrake (*Casarca cana*, Gm.), the Knob-Billed Duck (*Sarkidiornis melanotus*, Penn.), and the South African Pochard (*Nyroca capensis*), in the Rustenburg District, Transvaal.

3. *Trinoton aculeatum*, Piaget (1885).

Numerous specimens taken from White-faced Ducks or Tree Ducks (*Dendrocygna viduata*, Linné), in the Rustenburg District, Transvaal; also several specimens taken from the South African Sheldrake (*Casarca cana*, Gm.), the Knob-billed Duck (*Sarkidiornis melanotus*, Penn.), and the White-backed Duck (*Thalassornis leuconotus*, Eyt.) in the same district. This species was described by Piaget from specimens taken off a *Dendrocygna viduata*, in the Leyden Museum.

Genus PSEUDOMENOPON, Mjöberg.

Pseudomenopon, Mjöberg, Arkiv. f. Zoologi., vi, p. 50 (1910).

The collection contains several specimens taken from Moorhens, a Coot, a Cormorant, and a Snipe. Those taken from the Moorhens, a Coot, and a Cormorant appear to be intermediate forms between Kellogg's *P. insolens* and *P. pacificum*, the former of which was

described as a variety of *P. tridens*, Nitzsch, taken from an Eared Grebe (*Colymbus nigricollis californicus*) in the Bay of Monterey, California, and the latter also as a variety of *P. tridens*, Nitzsch, taken from an American Coot (*Fulica americana*) in the same district. For this reason I do not hesitate to sink *P. insolens* as a synonym of *P. pacificum*, K.

Those from a Snipe are a distinct species, for which I propose the name *P. rostratula*.

1. *Pseudomenopon pacificum*, Kellogg (1896).

Syn. *P. insolens*, Kellogg (1896).

This species differs from *P. tridens*, N., which has been found on Rails and Grebes in Europe, by the presence of six hairs instead of only two or four (according to Giebel, there are only four, and according to Piaget two) on the occipital margin; also by the lateral abdominal bands, which are dark brown instead of being uncoloured.

The following table gives the dimensions of the females of specimens in the collection from the different hosts, also those given by Kellogg:—

Host.	Total Length.	Length of Head.	Width of Head.	Width of Abdomen.
	mm.	mm.	mm.	mm.
1. <i>Gallinula chloropus</i>	1.89	0.29	0.53	0.7
2. <i>Gallinula angulata</i>	1.89	0.29	0.53	0.7
3. <i>Fulica cristata</i>	1.84	0.28	0.5	0.66
4. <i>Phalacrocorax lucidus</i>	1.78	0.28	0.51	0.68
5. <i>Fulica americana</i>	1.65	0.28	0.5	0.62
6. <i>Colymbus nigricollis</i>	2.00	0.31	0.53	0.72

The specimens from (1) *Gallinula chloropus* (Moorhen), (2) *G. angulata* (Lesser Moorhen), (3) *Fulica cristata* (Red-knobbed Coot), and (4) *Phalacrocorax lucidus* (White-breasted Ducker)* were all collected in the Rustenburg District, Transvaal, by Mr. Powell.

(2) *Pseudomenopon rostratula*, nov. sp.

Several females and males taken from *Rostratula capensis* (Painted Snipe) in the Rustenburg District, Transvaal, 22nd May, 1917 (W. Powell). This species closely resembles the other known species of the genus, but can be easily distinguished by the shape of the median lobe on the ventral surface of the head, which tapers to a point, instead of being broad and rounded at the apex. (Plate III, fig. 9.)

Female: Total length 1.71 mm.; length of head .28 mm.; width .5 mm.; width of prothorax .36 mm.; width of metathorax .46 mm.; width of abdomen .61 mm. Colour, pale yellowish-brown.

Head. Temples with four long bristles and some hairs; occiput with a narrow marginal band and six hairs; occipital bands very faint.

* This host was probably wrongly identified, and its true host was a species of *Podiceps*.

Prothorax: Much wider than long, with a short spine at each lateral angle; just beneath the spine there is a longish bristle, and beneath this a shorter one; inside the lateral angle, at each end of the transverse line, there is a short bristle; posterior margin with a row of ten hairs. *Metathorax*: With a row of hairs on the posterior margin.

The *abdomen* resembles that of *P. pacificum*, K., except that the lateral and transverse bands are light yellowish-brown; the lateral bands on the eighth segment and the whole of the ninth segment are, however, slightly darker than the rest of the abdomen.

Male: The male resembles the female, except that the lobes on the ventral surface of the head do not reach the posterior margin. Total length 1.32 mm.; length of head .21 mm.; width .36 mm.; width of prothorax .3 mm.; width of metathorax .35 mm.; width of abdomen .43 mm.

FAMILY LAEMOBOTHRIIDAE, Mjöberg.

Genus LAEMOBOTHRION, Nitzsch.

Laemobothrion, Nitzsch, Germar's Magazine, iii, p. 301 (1818).

1. *Laemobothrion kelloggi*, nom. nov.

Syn. *L. africanum*, Kellogg and Ferris (1915), nec Kellogg (1910).

One female taken off a Hadada Ibis (*Hagedaschia hagedasch*) at Mfongosi, Zululand (collected by Mr. Jones and presented by Mr. Chubb).

FAMILY TRICHODECTIDAE, Burmeister.

Genus TRICHODECTES, Nitzsch.

Trichodectes, Nitzsch, Germar's Magazine, iii, p. 294 (1818).

1. *Trichodectes bovis*, Linné (1758).

Syn. *T. scalaris*, Nitzsch (1838).

Numerous females and two males taken from cattle at Onderstepoort (G.A.H.B.).

2. *Trichodectes equi*, Linné (1758).

Syn. *T. parumpilosus*, Piaget (1880).

Numerous females taken from horses and donkeys at Onderstepoort. Mr. Hill has also found this species on a horse at Maritzburg. Fernandes has recorded it from Mozambique.

3. *Trichodectes pilosus*, Giebel (1874).

Syn. *T. equi*, Denny (1842), nec Linné (1758).

I have not been able to find this species in the Transvaal. Fernandes has recorded it having been taken on horses and mules in Mozambique.

4. *Trichodectes caprae*, Gurlt (1843).Syn. *T. climax*, Nitzsch (1861).

This species is very common on both Boar Goats and Angora Goats in South Africa.

5. *Trichodectes limbatus*, Gervais (1847).Syn. *T. crassipes*, Rudow (1866).

Several females and males taken from Angora Goats at Onderstepoort (G.A.H.B.) and at Maritzburg, Natal (A. L. Hill).

6. *Trichodectes ovis*, Linné (1758).Syn. *T. sphaerocephalus*, Olfers (1816).

Several females and males taken from sheep at Post Alma, Waterberg District, Transvaal (Van der Walt), and in the Pietersburg District, Transvaal (O. C. Weeber).

7. *Trichodectes cornutus*, Gervais (1847).Syn. *T. longiceps*, Rudow (1866).

Two females taken on a Mountain Reedbuck (*Cervicapra fulvorufula*) at Mfongosi, Zululand (collected by Mr. Jones and presented by Mr. Chubb).

8. *Trichodectes caffra*, nov. sp. (Plate III, figs. 10 and 11).

Female: Total length 1.22 mm.; length of head .34 mm.; length of prothorax .08 mm.; length of metathorax .05 mm.; length of abdomen .75 mm.; width of head .36 mm.; width of prothorax .28 mm.; width of metathorax .36 mm.; width of abdomen .6 mm.

Head: Very slightly wider than long. Forehead conical, emarginated at the apex, with sides slightly concave. Antennal bands broad and dark, separated in front by a small clear space. In front of each antenna there is a trabacula-like process. Antennal sinuses rather shallow. On each side of the forehead there are six hairs, two in front, three in the middle, and one above the antennal sinus. Ocular projection prominent, extending beyond the temples. Temples with a narrow dark marginal band, and three short hairs. Occipital margin straight. Occipital bands conspicuous, connected at their bases by a broad band, and meeting the antennal bands just in front of the trabacula-like processes. Antennae rather short and wide; the second segment the longest; third slightly longer than the first, with a sense organ.

Prothorax broader than long, with the posterior margin slightly convex. On each side there is a large conspicuous spiracle.

Metathorax as wide as the head, very short, with three or four short hairs on each side.

Abdomen elliptical, slightly longer than broad, with a median dark band on all the segments, except the first. On the posterior surface of each segment there is a row of a few very minute hairs.

Male: Total length 1.14 mm.; length of head .33 mm.; length of prothorax .08 mm.; length of metathorax .03 mm.; length of abdomen .70 mm.; width of head .35 mm.; width of prothorax .26 mm.; width of metathorax .35 mm.; width of abdomen .53 mm.

The *head* resembles that of the female, except that the antennal sinuses are much wider and deeper. Temples sparsely clothed with small hairs. It is quite possible that the temples in the female are also sparsely clothed with small hairs, but I cannot detect any in the type specimen.

Antennae: First segment long and wide, about as long as the second and third together, with two lines on the dorsal surface near the inner margin; second segment slightly longer than the third, with a small appendage at the base on the inner side; third segment with a sense organ situated in the middle of the segment, and two small spines at the apex.

Thorax and legs as in the female.

Abdomen oval, with median brown bands, and a row of minute hairs on the dorsal surface of each segment.

Genitalia as in Plate III, fig. 11.

Described from one female and one male taken off a Vaal Bush Cat (*Felis caffra*) at Block Spruit, Rustenburg District, Transvaal, 2nd April, 1916 (W. Powell).

9. *Trichodectes genetia*, nov. sp. (Plate IV, figs. 12 and 13).

Male: Total length 1.28 mm.; length of head .36 mm.; length of prothorax .08 mm.; length of metathorax .06 mm.; length of abdomen .78 mm.; width of head .36 mm.; prothorax .25 mm.; metathorax .31 mm.; abdomen .51 mm.

Head as long as wide. Forehead rounded, with four hairs on each side. Antennal band narrow, separated in front by a small clear space. Internal band conspicuous. In front of each antenna there is a trabacula-like process. Antennal sinuses wide and deep. Ocular projections small, with a small hair. Temples rounded, sparsely clothed with a few short hairs. Occipital margin very slightly convex. Occipital bands narrow, connected at their bases by a narrow band, and becoming indistinct towards the middle of the head. On the dorsal surface there is a transverse row of six hairs situated midway between the trabacula-like process and the anterior margin of the head; two more on each side just above the trabacula-like processes; two admedian hairs between the antennae, and two more situated almost in a line with the ocular projections. On the ventral surface there are four hairs on each side, situated just above the trabacula-like processes.

Antennae: First segment long and wide, not so long as the second and third segments together, with five hairs in a longitudinal line on the dorsal surface; second segment as long as the third, with a small appendage at the base on the inner side; third segment with a sense organ, and two small hooks at the apex.

Prothorax broader than long, but not so broad as the head, with the posterior margin very slightly convex. On each side there is a large conspicuous spiracle.

Metathorax short and broad, with four short lateral hairs on the posterior margin.

Abdomen oval, with a narrow, longitudinal band on the dorsal and ventral surface of each segment (in fig. 12 the bands on both the dorsal and ventral surfaces are depicted) and also a transverse row of minute hairs on both the dorsal and ventral surfaces.

Female (slightly immature): *Head* slightly broader than long. Forehead slightly acuminate in front. Antennal bands almost touching in front. Internal bands conspicuous in front, but become indistinct towards the base of the forehead. Antennal sinuses not so broad and deep as in the male. *Antennae* short and broad; first segment almost as broad as long, slightly longer than the second segment; the third segment a little longer than the first. Occipital bands narrow, and not connected at their base by a band, and meeting the antennal bands just above the antennal sinuses. In all other respects the female resembles the male, except that the median abdominal bands are absent, which is usually the case in immature specimens.

Described from two slightly immature females and one male taken off a Small-spotted Genet (*Genetta ludia*) at Jericho, Transvaal, 23rd March, 1916 (W. Powell).

This species appears to be closely related to *T. acuticeps*, Neumann, which was described from three females and three males taken off a genet in Abyssinia, but as Neumann's description is incomplete; and as he does not figure his species it is impossible to draw up an accurate comparison between his *T. acuticeps* and *T. genetta*. *T. genetta*, however, can be easily distinguished from *T. acuticeps* by the first antennal segment of the male, which is shorter than the two succeeding segments together in the former species, and as long as the two succeeding segments together in the latter species.

FAMILY PHILOPTERIDAE; Burmeister.

Genus GONIODES, Nitzsch.

Goniodes, Nitzsch, Germar's Magazine, iii. p. 293 (1818).

1. *Goniodes meleagridis*, Linné (1758).

Syns. *G. styliifer*, Nitzsch (1838).

G. styliiferum, Taschenberg (1882).

Males and females taken from a domestic turkey at Pretoria North, Transvaal, 24th April, 1917.

2. *Goniodes numidae*, Mjöberg (1910).

Several females and males taken from a Crowned Guinea-fowl (*Numida coronata*) at Bridgewater, Transvaal (W. Powell). This species appears to be closely related to *G. fimbriatus*, Neumann, which was described from five females and one male taken off an unknown host at Konakry, and from one female, also taken from an unknown host in Mozambique. It can, however, be distinguished by the lateral abdominal bands of the male being separated on their posterior margins.

Genus GONIOCOTES, Burmeister.

Goniocotes, Burmeister, Handbuch der Entomologie, ii, p. 431 (1838).

1. *Goniocotes gigas*, Taschenberg (1879).

Several specimens taken from domestic fowls at Onderstepoort and Maritzburg, Natal (A. L. Hill); also several females and males taken from a Crowned Guinea-fowl (*Numida coronata*) at Bridgewater, Transvaal, 10th June, 1916 (W. Powell).

2. *Goniocotes bidentatus*, Scopoli (1763).

Syn. *G. compar*, Nitzsch (1838).

Several females and males from domestic pigeons at Onderstepoort, 2nd May, 1914 (G.A.H.B.), and at Maritzburg, Natal (A. L. Hill).

Genus LIPEURUS, Nitzsch.

Lipeurus, Nitzsch, Germar's Magazine, iii, p. 292 (1818).

1. *Lipeurus caponis*, Linné (1758).

Syn. *Lipeurus variabilis*, Nitzsch (1838).

Several females and males taken from a domestic fowl at Maritzburg, Natal, June, 1917 (A. L. Hill).

2. *Lipeurus heterographus*, Nitzsch (1866).

Very common on fowls in the Transvaal and Natal.

Genus PHILOPTERUS, Nitzsch.

Philopterus, Nitzsch, Germar's Magazine, iii, p. 288 (1818).

Docophorus, Nitzsch, Germar's Magazine, iii, p. 289 (1818).

1. *Philopterus dentatus*, Scopoli (1763).*

Syn. *P. icterodes*, Nitzsch (1838).

Several females and males taken off the White-faced Duck (*Dendrocygna viduata*, Linné), the Knob-billed Duck (*Sarkidiornis melanotus*, Penn.),† and White-backed Duck (*Thalassornis leuconotus*, Eyt.) in the Rustenburg District, Transvaal; also two males taken off a domestic duck at Maritzburg, Natal (A. L. Hill).

2. *Philopterus cursor*, Nitzsch (1838).

Numerous specimens from a Spotted Eagle Owl (*Bubo maculosus*, Vieill) at Onderstepoort; also several specimens from a Bush Owl (*Syrnium woodfordi*, Gm.) in the Rustenburg District, Transvaal. Waterston has recorded this species from skins of *Bubo maculosus* and *Bubo capensis*, Gm. (the Cape Eagle Owl) in the South African Museum.

3. *Philopterus cerylinus*, Mjöberg (1910).

Several females and males taken from a Pied Kingfisher (*Ceryle rudis*, Linné) at Mooi Vlei, Transvaal, 26th August, 1916 (W. Powell).

P. cerylinus, Mjöberg, is most probably a synonym of *P. duplicatus*, Piaget, but as I have not been able to examine Piaget's description I am not in a position to state definitely whether it is or not.

* A new genus—*Anatoceus*—has recently been established by Cummings (Proc. Zool. Soc. Lond., p. 653, 1916) for the inception of this and six other species found on ducks, geese, and swans.

† A male from this host proves to be a specimen of *A. ferrugineus*, Giebel.

4. *Philopterus capistratus*, Neumann (1912).

Several females and males taken off a Brown-hooded Kingfisher (*Halcyon albiventris*) at Mooi Vlei, Transvaal, 26th August, 1916 (W. Powell), and from the same host at Maritzburg, Natal (A. L. Hill).

This species was described from two females taken off a *Halcyon semicaeruleus*, Rüpp.

The specimens in the collection differ from Neumann's in the breadth of the head, length of the abdomen, the presence of two admedian hairs in the middle of the first abdominal segment, and also by the presence of four admedian hairs on the ventral surface of the metathorax, and a row of hairs on the ventral surface of the first six abdominal segments.

In the specimens from which Neumann drew up his description there are no hairs on the ventral surface of the metathorax, nor are there any on the ventral surface of the second, third, and sixth abdominal segments. In the specimens in the collection there are two admedian hairs on the ventral surface of the first segment, six on the second, eight on the third, six on the fourth, and four on the fifth and sixth.

On the dorsal surface of the ninth segment there are two brown, lateral, triangular spots.

The male resembles the female, except that the head is as long as broad.

The following is a comparison of the measurements of Neumann's specimens and those in the collection:—

	From <i>H. semicaeruleus</i> .		From <i>H. albiventris</i> .			
	Female.		Female.		Male.	
	Length.	Breadth.	Length.	Breadth.	Length.	Breadth.
	mm.	mm.	mm.	mm.	mm.	mm.
Head.....	0·53	0·49	0·53	0·55	0·51	0·51
Thorax.....	0·27	0·45	0·28	0·48	0·25	0·46
Abdomen.....	0·96	0·63	1·11	0·68	0·93	0·63
TOTAL.....	1·78	—	1·93	—	1·69	—

I think there can be no doubt that the specimens in the collection are Neumann's species, although there are slight differences, because, judging by his measurements and figure of the ventral surface of a female, one is led to believe that he drew up his description from two immature females.

5. *Philopterus excisus*, Nitzsch (1838).

A single male taken from a House Martin (*Chelidon urbica*) at Jericho, Transvaal, 23rd March, 1916 (W. Powell).

Genus *DEGEERIELLA*, Neumann.

Degeeriella, Neumann, Bull. Soc. Zool. France, xx, p. 59 (1906).
Virmus, Nitzsch, Germar's Magazine, iii, p. 291 (1818), nec Hermann (1804).

Ricinus, Enderlein, Deutsche Sudpolar Exped., p. 447 (1909), nec De Geer.

1. *Degeeriella fusca*, Nitzsch (1842).

Syns. *D. appendiculata*, Piaget (1880).

D. vittata, Giebel (1874).

The collection contains numerous specimens of this common cosmopolitan species, which has been found on numerous falconiformes, from the African Goshawk (*Astur tachiro*, Daud), Maritzburg (A. L. Hill), the Black-shouldered Kite (*Elanus coeruleus*, Dels), Onderstepoort, 9th March, 1914 (G.A.H.B.), and the Lesser Kestrel (*Cerchneis naumannii*, Fleisch), Maritzburg (A. L. Hill).

2. *Degeeriella umbrina*, Nitzsch (1866).

Several females and males taken off the Hammerhead (*Scopus umbretta*) at Maritzburg (A. L. Hill) and in the Rustenburg District, Transvaal (W. Powell).

3. *Degeeriella hoplopteri*, Mjöberg (1910).

Several females and males taken from a Blacksmith Plover (*Hoplopterus speciosus*) at Bridgewater, Transvaal (W. Powell).

This species was described by Mjöberg from specimens taken off *Hoplopterus spinosus* in the Sudan.

4. *Degeeriella scolopacis*, Denny.

Syn. *D. truncata*, Nitzsch (1866).

One female taken off an Ethiopian Snipe (*Gallinago nigripennis*, Bp.) in the Pretoria District, October, 1897 (C. J. Swierstra).

5. *Degeeriella melanopheys*, Nitzsch (1866).

Syn. *Philopterus upupae*, Denny (1842), nec Schrank (1803). Two females and two males taken off an African Hoopoe (*Upupa africanus*) at Maritzburg, Natal (A. L. Hill).

Genus *ACIDOPROCTUS*, Piaget.

Acidoproctus, Piaget, Tijds. v. Ent., vi, p. 178 (1878).

1. *Acidoproctus bifasciatus*, Piaget (1878).

Numerous specimens taken off the following ducks in the Rustenburg District, Transvaal:— Red-billed Teal (*Anas erythrorhynchos*, Gm.), South African Sheldrake (*Casarca cana*, Gm.), White-faced Duck (*Dendrocygna viduata*, L.), Knob-billed Duck (*Sarkidiornis melanotos*), and the White-backed Duck (*Thalassornis leuconotus*, Eyt.); also one male from a Spur-winged Goose (*Plectropterus gambensis*, L.) taken in the same district.

2. *Acidoproctus stenopygus*, Nitzsch (1874).

Several females and males taken off a Spur-winged Goose (*Plectropterus gambensis*) in the Rustenburg District, Transvaal (W. Powell).

Genus *ESTHIPTERUM*, Harrison.

Esthipteron, Harrison, Parasitology, Vol. viii, pp. 26 and 129 (1916).

1. *Esthipteron struthionis*, Gervais (1847).*

Syn. *E. quadrimaculatum*, Piaget (1880).

This species is common on Ostriches (*Struthio australis*, Gurn.) at Onderstepoort. Piaget obtained his specimens from a North African Ostrich (*Struthio camelus*) and also from an American Ostrich (*Rhea americana*) in the Zoological Gardens at Rotterdam.

2. *Esthipteron crassicorne*, Scopoli (1763).

Syn. *E. squalidum*, Nitzsch (1842).

Several females and males taken off a Red-billed Teal (*Anas erythroryncha*) in the Rustenburg District, Transvaal (W. Powell).

3. *Esthipteron anseris*, Linné (1758).

Syns. *E. crassicorne*, Alfens (1816).

E. jejunum, Nitzsch (1842).

Several females and males taken off a domestic goose at Maritzburg (A. L. Hill).

4. *Esthipteron gambensis*, Piaget (1885).

Two females taken off a Spur-winged Goose (*Plectropterus gambensis*, L.) in the Rustenburg District, Transvaal.

5. *Esthipteron forficulatum*, Nitzsch (1866).

Several females and males taken off *Pelecanus rufescens*, Gm. (Pink-backed Pelican) in the Pretoria Zoo, 16th September, 1914 (C. J. Swierstra). This species was described by Nitzsch taken from *Pelicanus onocrotalus*. Kellogg has recorded it from *P. californicus* and *P. erythrorhynchus* in North America.

6. *Esthipteron ardeae*, Linné.

Syn. *E. leucopygum*, Nitzsch (1838).

Several females and males taken from a Grey Heron (*Ardea cinerea*, Linné) in the Rustenburg District, Transvaal.

7. *Esthipteron capitatum*, Piaget (1885).

One female taken from a Hadadah Ibis (*Hagedashia hagedash*) at Mfongosi, Zululand (collected by Mr. Jones and presented by Mr. Chubb).

8. *Esthipteron columbae*, Linné (1758).

Syn. *E. baculum*, Nitzsch (1866).

This species is very common on domestic pigeons in South Africa. The collection also contains numerous specimens taken from the Laughing Dove (*Turtur senegalensis*, Linné) and the Damara Turtle-Dove (*Turtur capicola damarensis*, Finsch and Hartl) in the Transvaal.

Waterston has recorded it taken from a stuffed Cape Fruit Pigeon (*Vinago delalandi*) in the South African Museum.

* Cummings has recently established the genus *Struthiolipeurus* (Proc. Zool. Soc., p. 679. 1916), for *E. rheae*, Harrison, and *E. struthionis*, Ger.

9. *Esthiopterum sudanicum*, Mjöberg (1910).

Several specimens taken from *Turtur senegalensis* and *Turtur capicola damarensis* in the Transvaal.

This species was described from specimens taken off a Red-eyed Turtle-dove (*Turtur semitorquatus*, Rüpp) in the Sudan. It is not nearly so common as *E. columbae*, which is a cosmopolitan parasite on pigeons.

EXPLANATION OF PLATES.

- Fig. 1.—*Polyplax waterstoni*, nov. sp. ♀.
 Fig. 2.—Pleural plates of female of *P. waterstoni*, nov. sp.
 Fig. 3.—Pleural plates of female of *P. arvicanthus*, nov. sp.
 Fig. 4.—♂ genitalia of *P. waterstoni*, nov. sp.
 Fig. 5.—Sternal plate of female of *P. waterstoni*, nov. sp.
 Fig. 6.—Sternal plate of female of *P. arvicanthus*, nov. sp.
 Fig. 7.—*Colpocephalum harrisoni*, nov. sp. ♀.
 Fig. 8.—Lobes on ventral surface of head of female of *Pseudomenopon pacificum*, Kellogg.
 Fig. 9.—Lobes on ventral surface of head of female of *P. rostratula*, nov. sp.
 Fig. 10.—*Trichodectes caffra*, nov. sp. ♀.
 Fig. 11.—♂ genitalia of *Trichodectes caffra*, nov. sp. (The suture between the two apical abdominal segments has been omitted, and the abdominal bands on the ventral as well as those of the dorsal surface are shown.)
 Fig. 12.—*Trichodectes genetia*, nov. sp. ♂.
 Fig. 13.—Head of female of *Trichodectes genetia*, nov. sp.
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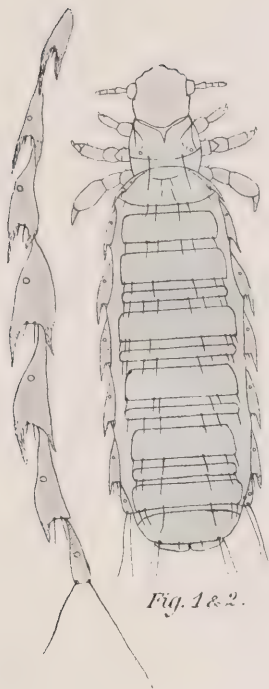


Fig. 1 & 2.



Fig. 3.



Fig. 4.



Fig. 5.



Fig. 6.

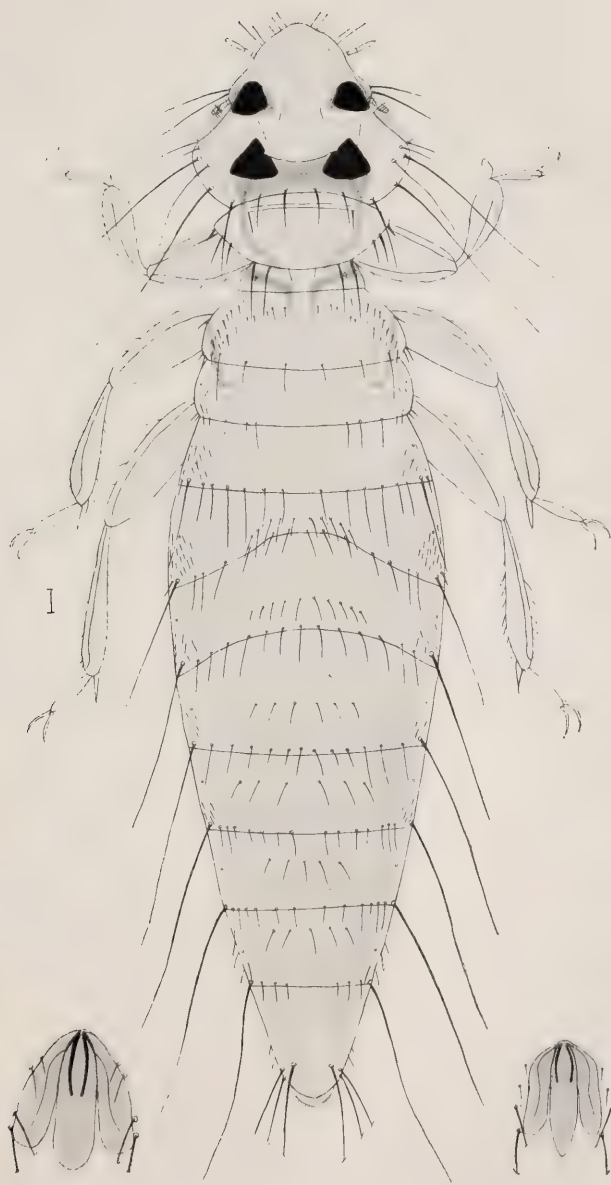


Fig. 8.

Fig. 7.

Fig. 9.

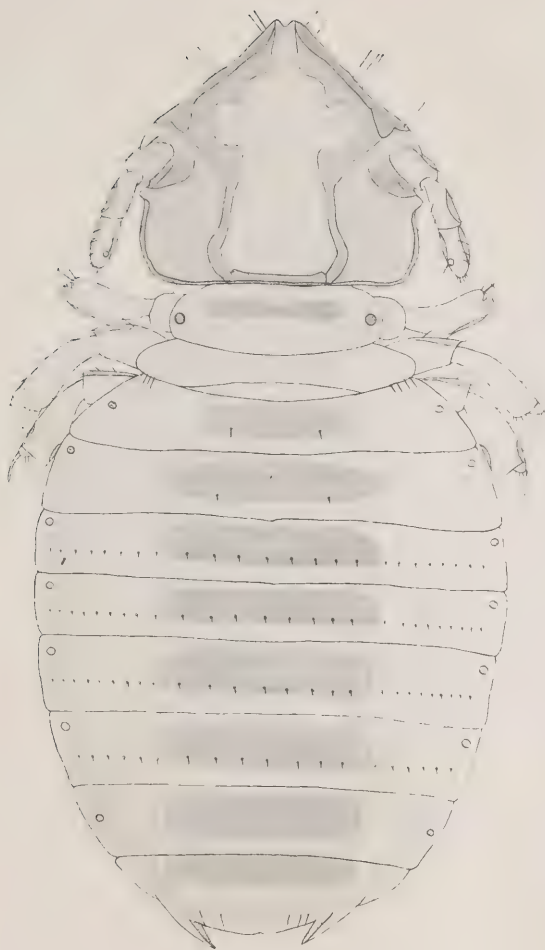
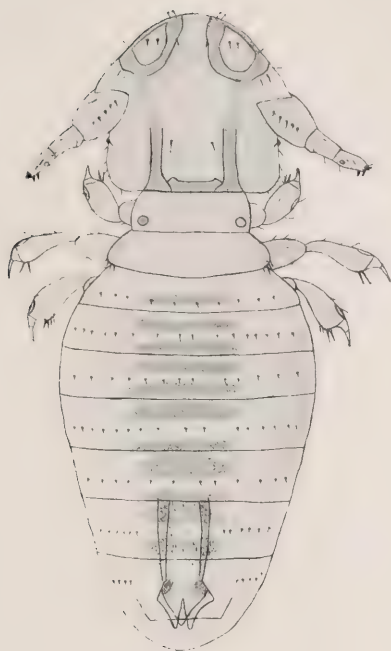


Fig. 10.



Fig. 11.

*Fig. 12.**Fig. 13.*

New Culicine Larvae from the Transvaal.

BY

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New Culicine Larvae from the Transvaal.

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THE material upon which the present paper is based was collected at Onderstepoort, near Pretoria. The drawings were made with the aid of a Zeiss binocular microscope to which was attached a *camera lucida*.

For further information on the Culicine larvae of Africa the reader may be referred to the following papers:—

Hill and Haydon.—A contribution to the Study of the Characteristics of Larvae and Species of Anophiline in South Africa. *Annals of the Natal Government Museum*, Vol. I. pt. 2, pp. 111-157, 1907.

Welché, W.—On the Larval and Pupal Stages of West African Culicidae. *Bull. of Ent. Res.*, Vol. I, pt. 1, pp. 7-50, 1910.

Edwards, F. W.—Revised Keys to the Known Larvae of African Culicinae. *Bull. of Ent. Res.*, Vol. III, pt. 4, pp. 373-385, 1912.

Scott Macfie and Ingram.—New Culicine Larvae from the Gold Coast. *Bull. of Ent. Res.*, Vol. VII, Part 1, pp. 1-18, 1916.

Sub-Family *Culicinae*.

Tribe *Culicini*.

Mucidus scatophagoides, Theobald.

The larvae during life are pale yellowish in colour with reddish-brown eyes.

The *head* is large, and on each side in front, in place of the usual feeding brushes, there is a large chitinized appendage. These appendages are brown at the base and black at the apex, and when viewed under a high-powered lens show minute pectination at the apices. The antennae are short, slender, and are not constricted in the middle; the hair-tuft is situated on the dorsal surface near the apex and consists of three short hairs. On each side of the head in front there is a short simple hair, also one on each side near the base of the antennae, and two in the middle of the head.

The *thorax* is large; in front there are two long hairs on the dorsum, each of which has two simple branches; the remainder of the hairs and plumes are all simple.

The *abdomen* has simple plumes on the lateral margins of the first three segments.

The *comb* consists of about 30-35 spines. The siphonal, sub-siphonal, and anal plumes consist of simple hairs.

The *anal segment* is about as broad as long, and has four long hairs on the dorsum and a well-developed ventral brush; on the posterior margin there is a row of minute spines. The *papillae* are slightly shorter than the anal segment.

The siphon is five times the length of the diameter of its base, and has about twenty-four spines in the pecten, beyond which is a tuft of four or five hairs.

Breeding places.—The larvae were found in a rain-water pool last February.

The habits of the larvae were not observed as they were fully mature when collected, but I think there can be no doubt, judging by the appendages on the head, that they are parasitic upon other larvae.*

In life they might easily be mistaken for the larvae of *C. tigripes* at first sight, but can at once be differentiated from that species by their long, slender siphon. This species is by no means common at Onderstepoort.

According to Edwards *all* the tarsi of the adults of this species have pale basal bands, most marked on the hind pair. However, in the type *Sudanensis* (which Edwards sinks as a synonym of this species) and the specimens bred out here, all the metatarsi have, in addition to the basal bands, a median white band, and the last two joints of the fore and mid tarsi are entirely yellow.

The number of white spots on the wing-fringe varies from seven to eight, sometimes even in the wings of the same individual.

Ochlerotatus hirsutus, Theobald.

The larvae in life are light grey in colour. The head is brown and often of a slightly darker colour round the anterior margin. The siphon is light brown in colour.

The *head* is rather small with antennae of normal size. There is no constriction in the middle of the antennae at the insertion of the hair-tuft. The hair-tuft consists of six to eight simple hairs. The outer pair of median frontal hairs are longish and subplumose; the inner pair are short and consist of five branches of simple hairs.

The *thorax* is of medium size; in front on the anterior margin there are two small stellate hairs. The hairs on the lateral margins are all subplumose, except one long hair on each side which is simple.

The *abdomen* has subplumose hairs on the lateral margins of the first six segments. On the first and second segments there are two admedian plumes consisting of short, simple hairs; those of the first segment being slightly longer than those of the second.

The *comb* is formed of about fifteen spines; immediately behind the comb is a short stellate hair consisting of four branches. The

* Since the above was written more specimens were obtained in January and February, 1919. They were found in mud pools and marshy ground along with larvae of *Ochlerotatus hirsutus*, Theob., and *Banksinella lineatopennis*, Ludlow, upon which they are predaceous.

siphonal, subsiphonal, and anal plumes are composed of subplumose hairs.

The *anal segment* is slightly longer than broad; the hairs on the dorsum consist of one long hair and a tuft of strong hairs; the ventral brush is well developed; at the apex there are numerous minute spines.

The *siphon* is about three times as long as it is broad at its base; there are about thirty-two spines in the pecten; the last two widely separated, and between them there is a small hair-tuft.

Breeding places.—The larvae have been found in pools on roads after heavy rains, and in holes on the margins of streams and ponds made by horses' hoofs. They have also occasionally been found in holes in the ground containing a foot or two of water and free of vegetation.

Both larvae and adults have only been collected during the summer months.

Ochleratatus argenteopunctata, Theobald.

The *head* is small with longish antennae; there is no constriction in the middle of the antennae at the insertion of the hair-tuft; the hair-tuft consists of four simple hairs. There are four frontal median hairs of equal length, each hair consisting of four branches, which are plumose.

The *thorax* is small. In front there are two long hairs on the dorsum, each of which has two subplumose branched hairs. The hairs on the lateral margins are also subplumose.

The *abdomen* has plumes on the first two segments. Each plume only consists of three simple hairs. Segments III-VI each with a long simple hair on the lateral margins. The *comb* is formed of about fourteen to twenty spines arranged more or less in two rows. The subsiphonal and anal plumes are composed of subplumose hairs.

The *anal segment* is as broad at the apex as what it is long, with long hairs on the dorsum and a ventral brush which consists of short hairs, except for a few at the apex which are as long as the papillae. The *anal papillae* are narrow and nearly four times as long as the anal segment.

The *siphon* is nearly four times as long as what it is broad at the base. There are eight longish thorn-like spines in the pecten, the last two of which are widely separated, and between them is situated a tuft of four long hairs, which are subplumose.

Breeding places.—The larvae have been found in pools along the banks of the Aapies River at Onderstepoort during the summer months.

Culex tipuliformis, Theobald.

In life the larvae are light in colour.

Head moderately large. The antennae have the distal third darkened, also a narrow dark band at the base, and are covered with spicules; the hair-tufts are situated at two-thirds the length of the antennae and are formed of plumose hairs. The mid-frontal hairs each consist of three branches, which are plumose.

All the hairs on the thorax are plumose.

The lateral hairs on the first four abdominal segments are plumose, those on the remaining segments simple.

The *comb* consists of thirty to forty spines arranged in a triangular patch. The hairs on the siphonal and subsiphonal plumes are plumose, and those on the anal plume simple.

The *anal segment* is longer than what it is broad at the base, with a well-developed ventral brush, and three very long and five longish hairs on the dorsum. The *anal papillae* are slightly shorter than the anal segment.

The *siphon* is long and narrow, seven times the length of its diameter at the base and slightly less than half the length of the abdomen. The *pecten* is formed of about nine simple spines; beyond these there are about ten ventral tufts composed of simple hairs.

Breeding places.—The larvae of this extremely common mosquito have only been found in streams at Onderstepoort.

Culex salisburyensis, Theobald.

The larvae in life are pale in colour with a large dark spot on the posterior margin of the thorax and a dark median line extending along the whole length of the abdomen.

The *head* is small. The antennae are long and have the distal third darkened, also a narrow dark band at the base, and are covered with spicules; the hair-tufts are situated at two-thirds the length of the antennae, and are formed of plumose hairs. There are four simple mid-frontal hairs. The hairs on the anterior and lateral margins of the prothorax are all simple; the plumes, of which there are four on each lateral margin, are composed of plumose hairs.

The plumes on the first three abdominal segments are composed of plumose hairs; those on segments IV-VI of simple hairs.

The *comb* is formed of about forty to fifty spines, arranged in a triangular patch. The hairs of the subsiphonal and siphonal plumes are plumose, and those of the anal plume simple. The anal segment is nearly twice as long as what it is broad, with three long and three or four short hairs on the dorsum; the ventral brush is well developed. The anal papillae are small and of equal length.

The *siphon* is long and narrow, about nine times the length of its diameter at the base, and slightly less than half the length of the abdomen. There are about fourteen spines in the pecten, which are situated close together; beyond these there are eight to twelve ventral hair tufts composed of simple hairs.

Breeding places.—The larvae have been found in pools along the banks of the Aapies River and in streams at Onderstepoort, but they are not often found in the latter.

NOTE ON THE BREEDING HABITS OF
CULEX DECENS, THEOBALD.

Larvae of this species were submitted to the author by Mr. C. J. Brain, who collected them in a coal mine 300 feet below the surface of the ground at Witbank, Transvaal.

Dr. Graham records finding an egg-raft of this species upon water in a tin containing vegetable matter at Lagos, West Africa.

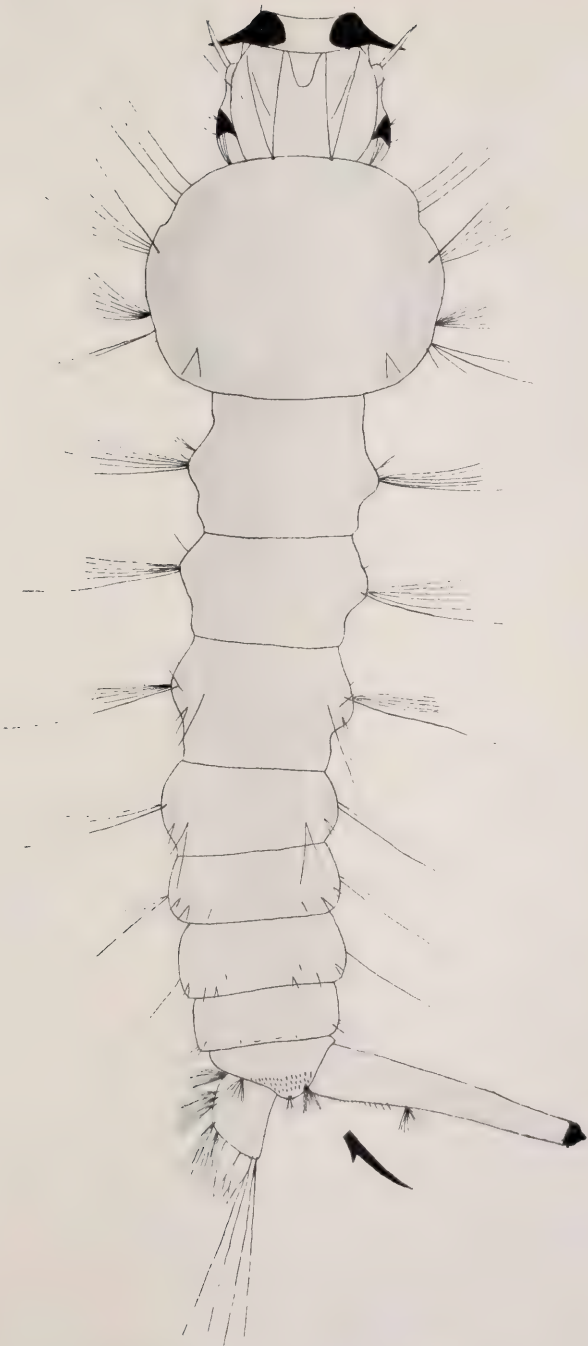


FIG. 1.—*Mucidus scatophagoides*, Theobald.

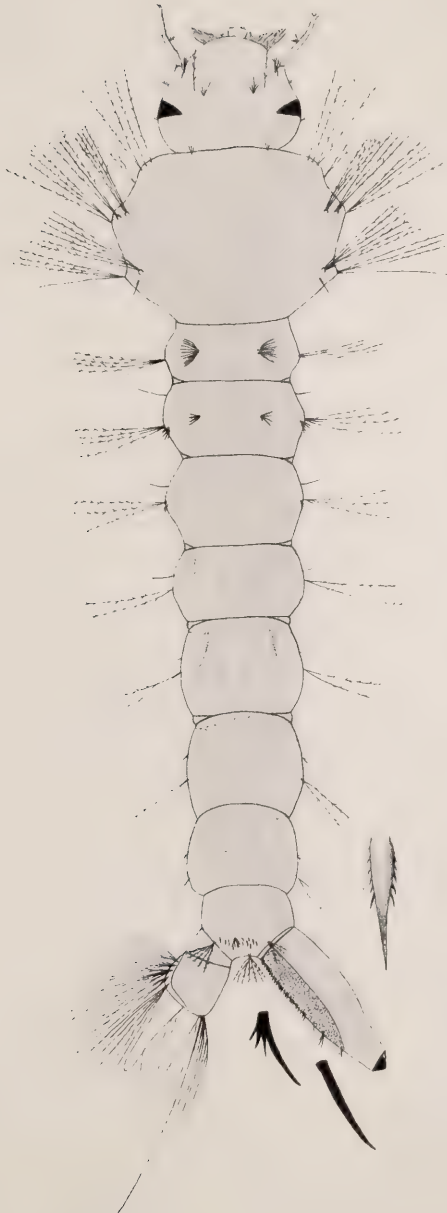


FIG. 2.—*Ochlerotatus hirsutus*, Theobald.

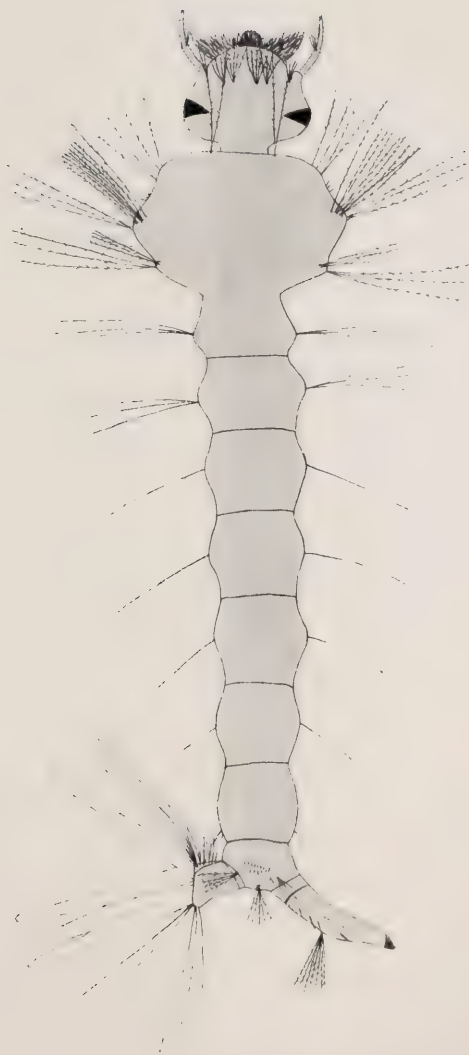


FIG. 3.—*Ochlerotatus argentopunctata*, Theobald.

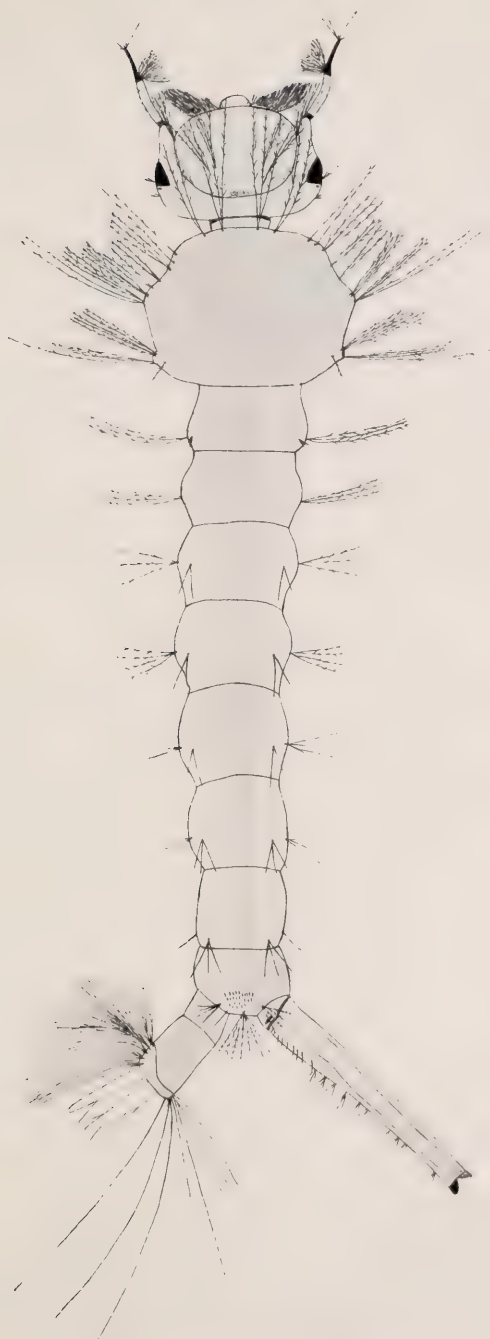


FIG. 4. *Culex tipuliformis*, Theobald.

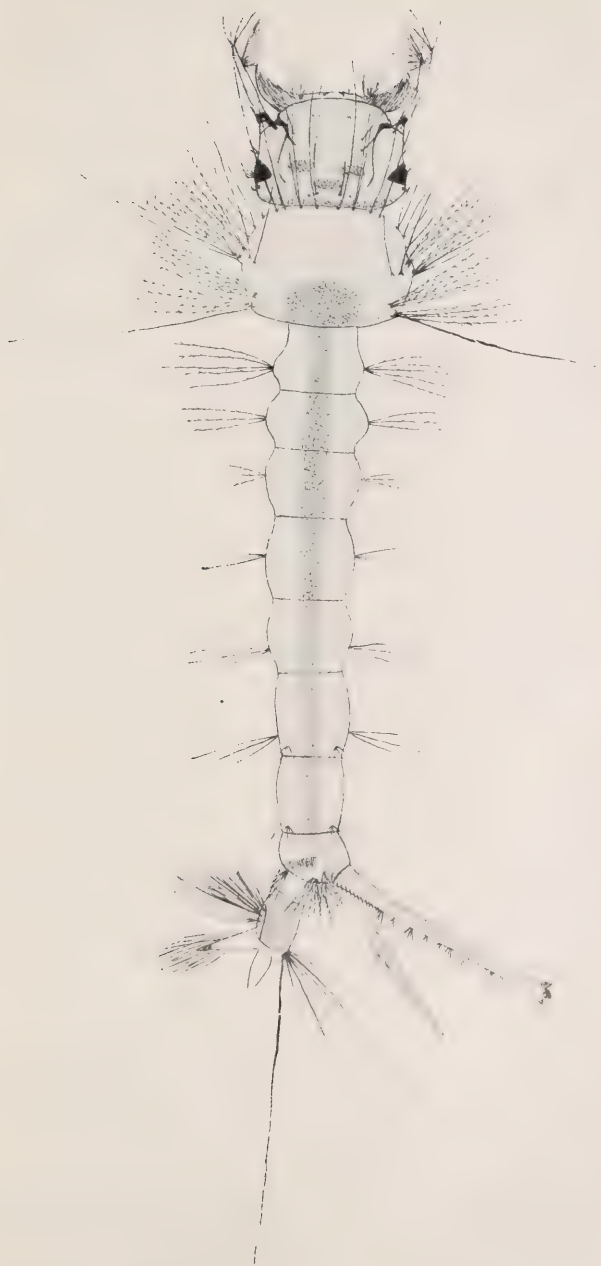


FIG. 5. *Culex salishuriensis*, Theobald.

The Deficiency Aspect of Maize Products.

BY

HENRY H. GREEN, D.Sc.,

Biochemist, Division of Veterinary Research.

From the Onderstepoort Laboratories for Veterinary Research.

Pretoria, September, 1917.

The Deficiency Aspect of Maize Products.

By HENRY H. GREEN, D.Sc., Biochemist, Division of Veterinary Research, Onderstepoort.

(Received September, 1917.*)

At the time we were engaged in determining the anti-neuritic value of certain foodstuffs fed to cattle in connection with experiments upon Lamziekte,¹ a few maize products were included. It was found that "samp" or "pearl hominy," i.e. mealies from which the outer pericarpal layers have been removed by mechanical rasping, was notably deficient in "vitamine" or "antineuritic hormone," but that the ordinary commercial maize meals were not only efficient when fed as sole diet to pigeons, but usually contained the antineuritic hormone in considerable excess over minimum avian requirements. A series of determinations of the relative antineuritic values of different maize milling products was then begun.

At this time a paper by McCrae² appeared in which analyses of the phosphoric oxide content of variously milled maize meals were given, and in which, on the basis of private information from Dr. D. Macaulay, it was stated that the incidence of deficiency disease (scurvy or a scurvy-like form of beri-beri) amongst the native labourers on the Johannesburg mines was lower where slightly milled meal was used than it was where the meals were milled in the ordinary way.

Although our own dietetic tests with pigeons had shown that the antineuritic vitamine of maize, like the vitamine of rice, was located mainly in the outer layers of the grain, and in its distribution appeared to follow the distribution of the phosphoric oxide, so evidencing the general accuracy of McCrae's assumption of parallelism, we were nevertheless led to conclude that a determination of phosphoric oxide in itself was no guide to the quantity of vitamine present, and that the customary mode of milling maize for native consumption would

* Read before the South African Association for the Advancement of Science, July, 1917, and, by permission of the Director of Veterinary Research, printed in abstract in the *South African Journal of Science*.

¹ Theiler, Green, and Viljoen: "Contribution to the Study of Deficiency Disease, with Special Reference to the Lamziekte Problem in South Africa." Third and Fourth Reports of the Director of Veterinary Research, Union of South Africa.

² J. McCrae: *J. Hyg.*, XIV, 3, Nov. 13, 1914. "The Phosphoric Oxide Content of Maize Flour."

not be likely to bring the average product used on the mines down to the danger line of actual deficiency.

We had already, at the request of Dr. Orenstein, Superintendent of Sanitation, Rand Mines, Limited, examined a few stock meals used for native rations, but these showed a very fair margin of safety when tested on pigeons. Dr. Orenstein also informed us that the statistical evidence available upon the relationship between the incidence of scurvy and the extent of milling of the meal used was too inadequate to serve as a basis for any valid conclusion. Indeed, since beri-beri as distinct from scurvy has not yet been clearly diagnosed on the Rand, and since it is not improbable that the antiscorbutic vitamine and the antiberi-beri vitamine are physiologically different entities, it is questionable whether the appearance of scurvy can be connected with the maize moiety of the native ration at all. It seems more probable that the source of the antiscorbutic vitamine is dependent upon the nature and quantity of the non-maize supplementary ration, of which a very fair amount is issued,³ and this is in harmony with Orenstein's observation⁴ that scurvy does not appear so long as the customary allowance of vegetables or fruit is not cut down.

The problem raised, however, is of great economic importance. Since maize is the staple cereal in so many parts of the world, and since the relationship between the different clinically recognized deficiency diseases is far from clear, it is important that the antineuritic values of maize products should be known. Since the simple and convenient determination of phosphoric oxide has been repeatedly maintained to be a guide to the antineuritic value, it is important that the relationship between the two should be clearly defined. Thus, in the United States Public Health Reports of 14th April, 1916, it is suggested for maize flour that "the P_2O_5 content should not be less than 0.50 per cent." As will be shown presently, such a health standard would rule out the bulk of South African maize, lead to serious error of judgment, and be impossible of general application.

Since the pigeon is the most useful experimental subject upon which to test a diet in respect to its antineuritic or antiberi-beri hormone, it is of the greatest importance that the relationship between human and avian requirements should be accurately known. Unfortunately this latter relationship is not one which can be readily determined experimentally—for obvious reasons. At the same time the data accumulated by medical workers in various parts of the world already provide us with approximate information in this direction, so that it is possible to translate results derived from pigeon tests into results available for human dietetics with a fair degree of accuracy. It must of course be emphasized that this only holds for deficiency in beri-beri vitamine; in respect to deficiency in other directions human and avian requirements appear to be widely different.

The method adopted in our pigeon tests was the now classical method of feeding known quantities of the diet to be tested mixed with fixed quantities of some standard basal deficient diet and so determining the minimum amount required to protect against *Polyneuritis gallinarum*. Various investigators have selected various

³ A. J. Orenstein: Annual Report of the Department of Sanitation for the Year 1915, Rand Mines, Ltd.

⁴ Same report for the year 1916.

intervals of time before concluding the test. Cooper,⁵ for instance, adopted an experimental period of fifty days. Our own experience, however, indicates this as rather short, and in most of our tests a minimum duration of four months was observed. Survival for this period is, in the case of the pigeon, as a rule sufficiently lengthy to be regarded as "infinite." If a bird remains healthy, and has not lost seriously in weight after feeding for 100 to 120 days on any diet, it is rare to find it succumbing to polyneuritis no matter how much longer the feeding be continued. At any rate the diet is then indicated as being at least on the border-line of efficiency.

In the first series of experiments, polished rice, the conventional deficient basal diet, was used as diluent. From certain evidence subsequently obtained it appeared that even highly polished rice still contained considerable quantities of vitamine, and hence, since it was desired to express the data for maize independently of a non-maize base, a second smaller series of tests was carried out in which samp, of very low phosphoric oxide content, was used as basal ration.

The following list represents the general series, along with the determinations of the phosphoric oxide contained:—

A. Whole maize	P_2O_5	.59 %
B. Fine meal	P_2O_5	.46 %
C. Seconds	P_2O_5	.65 %
D. Bran	P_2O_5	.67 %
E. Samp	P_2O_5	.12 %
F. Hominy chop, the complete by-product from the samp machine	P_2O_5	1.42 %
G. Mealie flour	P_2O_5	.59 %
H. Special table meal	P_2O_5	.34 %
I. Table meal	P_2O_5	.26 %
J. Specially prepared table meal	P_2O_5	.38 %
K. Fanko, a flake or rolled table product ...	P_2O_5	.16 %
L. Native meal, No. 1, supposed to contain no ground husks, and in use on four mines of the Rand group	P_2O_5	.46 %
M. Native meal, No. 3, supposed to contain a variable amount of husk, and in use on ten mines of the Rand group	P_2O_5	.48 %
N. Mealie meal "all in," a meal supposed to consist of whole grain after preliminary cleaning	P_2O_5	.36 %
O. A yellow mealie meal	P_2O_5	.17 %
P. Large white mealies ground in the Laboratory	P_2O_5	.66 %
Q. Small white mealies ground in the Laboratory	P_2O_5	.45 %

⁵ Cooper: "On the Protective and Curative Properties of Certain Foodstuffs against Polyneuritis induced in Birds by a Diet of Polished Rice." *Journ. Hygiene*, Vol. XII, 1912.

R. Maize germ meal, similar to hominy chop, but supposed to contain less bran	P_2O_5	1.41 %
S. Germ meal of different origin to "R" ...	P_2O_5	1.81 %
T. Hominy chop of different origin to "F" ...	P_2O_5	1.62 %
U. Samp of different origin to "E"	P_2O_5	.090 %

Samples A to D were stated to be from one milling, and E and F to be derived from A. In the making of samp the proportion of original grain converted into samp depends upon the quality of the maize. With a "No. 1" quality the proportions run about 62 per cent. of samp and 38 per cent. of hominy chop. Samples P and Q were from the same bag, sorted by hand into three sizes of grain and discarding the intermediate material. 100 gm. by weight consisted of about 170 kernels for P and about 310 for Q. It had been expected that the smaller grains with the relatively larger pericarpal area in proportion to endosperm would have been richest in phosphorus, but the reverse appears to be the case.

Method.—In carrying out the tests, pigeons were used in triplicate (three birds to a cage in each case). The birds were first starved for twenty-four hours, and so weighed with empty crops. To the basal diet of highly polished rice the various maize products, passed through a coffee mill if not already fine enough, were added in varying quantity in such a way that the gross food for each cage corresponded to one-twentieth of the body weight of the birds concerned. Thus, if the three birds in a cage weighed 285 gm., 290 gm., and 305 gm. respectively, totalling 880 gm., the daily food for the cage would be 44 gm. If it were desired to supply each bird with 6 gm. of mealie flour the cage diet would be prepared by weighing out 18 gm. of this and 26 gm. of polished rice, mixing, working into a stiff dough with hot water, dividing at sight into three portions, and feeding to each bird by hand. The error of sight division averages itself out every few days, and is permissible when the body weights of the three pigeons in the same cage do not differ too widely. Compulsory feeding is necessary, however, since birds left to regulate their own intake may voluntarily reduce their ration to a few gm. a day as soon as deficiency begins to be felt, and so complicate results. In general, the birds were small in build, the weight ranging around 300 gm. (crop empty), and the gross daily intake from 13 to 17 gm. Minor improvements in the method of carrying out the tests, such as feeding mixtures on a round percentage basis, suggested themselves in the course of the work, but in this first rice-maize series no actual change in procedure was made. The testing of the products enumerated was carried out over a period of about eighteen months as exigencies of other work permitted, but care was taken to keep results as comparable as possible. Since each separate batch of tests involved daily feeding of many of the birds for four months or more, a complete record of data and observations would involve a degree of prolixity intolerable to the general reader, and the main bulk of the tests may therefore be fused together and cast into the form of a consecutive table of results. Tests which proved irrelevant after completion, such as those on mixtures of rice and maize table meals which turned out to be deficient in themselves, are omitted. The cages are numbered consecutively for convenient

reference in the text, but in any given cage the three birds are always experimental fellows, dieted at the same time. In the fourth column the phosphoric oxide content of the diet is given to two significant places, since the method of experimentation renders closer expression pointless. The diagnoses were made from general experience of the symptoms, which in most cases were unmistakable. Many birds dying overnight are entered up as "polyneuritis" on the evidence of the symptoms of preceding days. In some of the obscure chronic cases diagnoses were confirmed by injection of vitamine extract, or by nursing back to health on a vitamine-rich diet. Wherever inter-current disease was suspected in any cage all three birds were discarded and a fresh cage put up.

It may also be mentioned that the birds used, though put up in different batches at different times, were all on a diet of whole maize *ad lib.* in the storing run for several weeks or months before going into experiment. Their preliminary dietetic treatment, if this has much influence, may therefore be regarded as fairly uniform throughout.

In Table I the results on 147 birds are recorded:—

TABLE I.

Cage.	Bird.	Diet.	Percentage P_2O_5 in Diet.	Fate.	Survival in Days.
1	1 2 3	Polished rice alone	.22	Polyneuritis " "	17. 15. 24.
2	4 5 6	Whole maize "A" alone	.59	Remained healthy " "	All three carried over 120 days.
3	7 8 9	Polished rice, plus 2 grm. maize "A" per bird	.26	Polyneuritis " "	28. 24. 37.
4	10 11 12	Polished rice, plus 3 grm. maize per bird	.28	Polyneuritis " "	35. 45. 39.
5	13 14 15	Polished rice, plus 4 grm. maize "A" per bird	.31	Polyneuritis " Chronic polyneuritis	50. 80. 105.
6	16 17 18	Polished rice, plus 5 grm. maize "A" per bird	.35	Remained healthy " "	All carried over 120 days.
7	19 20 21	Polished rice, plus 6 grm. maize "A" per bird	.37	Polyneuritis Remained healthy "	80. Over 200 days.
8	22 23 24	Polished rice, plus 7 grm. maize "A" per bird	.40	Remained healthy " "	All carried over 120 days.

TABLE I—(continued).

Cage.	Bird.	Diet.	Percentage P_2O_5 in Diet.	Fate.	Survival in Days.
9	25 } 26 } 27 }	Fine meal "B" alone	.46	Remained healthy " "	All carried over 200 days.
10	28 } 29 } 30 }	Polished rice, plus 3 grm. fine meal "B" per bird	.24	Polyneuritis " "	22. 20. 29.
11	31 } 32 } 33 }	Polished rice, plus 4 grm. fine meal "B" per bird	.29	Polyneuritis " "	40. 35. 61.
12	34 } 35 } 36 }	Polished rice, plus 6 grm. fine meal "B" per bird	.33	Chronic polyneuritis " Healthy	85. 62. Over 120 days.
13	37 } 38 } 39 }	Polished rice, plus 2 grm. seconds "C" per bird	.28	Polyneuritis " Chronic polyneuritis	44. 38. 72.
14	40 } 41 } 42 }	Polished rice, plus 4 grm. seconds "C" per bird	.35	Remained healthy " "	All carried over 120 days.
15	43 } 44 } 45 }	Polished rice, plus 2 grm. bran "D" per bird	.28	Polyneuritis " "	75. 75. 63.
16	46 } 47 } 48 }	Polished rice, plus 4 grm. bran "D" per bird	.34	Remained healthy " "	All carried over 120 days.
17	49 } 50 } 51 }	Samp "E" alone	.12	Polyneuritis " "	10. 9. 14.
18	52 } 53 } 54 }	Polished rice, plus 1 grm. hominy chop "F" per bird	.31	Polyneuritis Chronic polyneuritis Remained healthy	90. 106. Over 120.
19	55 } 56 } 57 }	Polished rice, plus 2 grm. hominy chop "F" per bird	.38	Remained healthy " "	All carried over 200 days.
20	58 } 59 } 60 }	Polished rice, plus 3 grm. mealie flour "G" per bird	.30	Polyneuritis " "	50. 40. 71.
21	61 } 62 } 63 }	Polished rice, plus 5 grm. mealie flour "G" per bird	.35	Polyneuritis Remained healthy "	68. Carried over 120 days.
22	64 } 65 } 66 }	Polished rice, plus 7 grm. mealie flour "G" per bird	.39	Remained healthy " "	All carried over 120 days.

TABLE I—(continued).

Cage.	Bird.	Diet.	Percentage P_2O_5 in Diet.	Fate.	Survival in Days.
23	67 68 69	Special table meal "H" alone	.34	Chronic polyneuritis " " Remained healthy	98 69. Over 120.
24	70 71 72	Table meal "I" alone	.26	Polyneuritis " " "	20. 25. 31.
25	73 74 75	Special table meal "J" alone	.38	Polyneuritis " " "	19. 16. 16.
26	76 77 78	Fanko "K" alone	.16	Polyneuritis " " "	18. 15. 16.
27	79 80 81	Native meal No. 1 alone	.46	Remained healthy " " "	Carried over six months with gain in weight.
28	82 83 84	Polished rice, plus 3 grm. native meal No. 1	.27	Polyneuritis " " "	54. 34. 65.
29	85 86 87	Polished rice, plus 8 grm. native meal No. 1, per bird	.36	Remained healthy " " "	Carried over 120 days with gain in weight.
30	88 89 90	Native meal No. 3 alone	.48	Remained healthy " " "	Put out of experi- ment after six months with gain of weight.
31	91 92 93	Polished rice, plus 3 grm. No. 3 native meal, per bird	.28	Chronic polyneuritis " " Acute polyneuritis	79. 96. 33.
32	94 95 96	Polished rice, plus 6 grm. No. 3 native meal, per bird	.37	Remained healthy " " "	Put out after 150 days.
33	97 98 99	Mealie meal "All in" "N" alone	.36	Remained healthy " " "	Carried over 120 days.
34	100 101 102	Polished rice, plus 3 grm. meal "N," per bird	.25	Polyneuritis " " "	29. 31. 38.
35	103 104 105	Polished rice, plus 6 grm. meal "N," per bird	.28	Polyneuritis Remained healthy "	79 Carried over 200 days.
36	106 107 108	Yellow mealie meal "O" alone	.17	Polyneuritis " " "	13. 21. 31.

TABLE I—(continued).

Cage.	Bird.	Diet.	Per- centage P_2O_5 in Diet.	Fate.	Survival in Days.
37	109 } 110 } 111 }	Polished rice, plus 3 grm. large mealies "P," per bird	.30 {	Polyneuritis " "	40. 41. 61.
38	112 { 113 { 114 }	Polished rice, plus 4 grm. large white mealies "P," per bird	.32 {	Polyneuritis " "	50. 79. Over 120.
39	115 } 116 } 117 }	Polished rice, plus 5 grm. mealies "P," per bird	.36 {	Remained healthy " "	Carried over 120 days.
40	118 { 119 { 120 }	Polished rice, plus 3 grm. small white mealies "Q," per bird	.26 {	Polyneuritis " "	33. 32. 33.
41	121 } 122 } 123 }	Polished rice, plus 4 grm. mealies "Q," per bird	.28 {	Polyneuritis " "	55. 54. 19.
42	124 } 125 } 126 }	Polished rice, plus 6 grm. mealies "Q," per bird	.31 {	Remained healthy " "	Carried over 120 days.
43	127 } 128 } 129 }	Polished rice, plus 8 grm. mealies "Q," per bird	.33 {	Remained healthy " "	Carried over 120 days.
44	130 } 131 } 132 }	Polished rice, plus 1 grm. maize germ meal "R," per bird	.34 {	Remained healthy " "	Carried over 120 days.
45	133 } 134 } 135 }	Polished rice, plus 1 grm. germ meal "S," per bird	.38 {	Remained healthy " "	Carried over 120 days.
46	136 } 137 } 138 }	Polished rice, plus 1 grm. hominy chop "T," per bird	.29 {	Polyneuritis " Chronic polyneuritis	67. 48. 95.
47	139 } 140 } 141 }	Polished rice, plus 1.5 grm. hominy chop "T," per bird	.35 {	Chronic polyneuritis Remained healthy "	98. Put out after five months.
48	142 } 143 } 144 }	Polished rice, plus 2 grm. hominy chop "T," per bird	.40 {	Remained healthy " "	Carried over 120 days.
49	145 } 146 } 147 }	Samp "U" alone	.09 {	Polyneuritis " "	9. 13. 14.

Surveying this table, it is at once strikingly apparent that the highly milled table meals and specially prepared table products are

all deficient. Thus samp, fanko, and two of the three table meals are markedly deficient, and produce acute polyneuritis in the brief period of nine days to one month. The third sample of table meal ("H") appears to lie just on the border-line of efficiency, one of the three birds remaining healthy and the other two succumbing after two to three months' feeding. Yellow meal ("O"), though not stated as highly milled, appears to have been so.

The other extreme is represented by germ meal and hominy chop with a vitamine content so high that one to two grm. (mixed with 13 to 16 grm. of polished rice) sufficed to protect indefinitely. In between come the ordinary milled meals, and maize bran. No meal, however, unless regarded by the manufacturers as a highly milled table product is actually shown to be deficient. The two native meals can both stand dilution with an equal weight of polished rice before a deficient mixture is produced.

Surveying the results as a whole, the vitamine content seems to follow the phosphoric oxide content with considerable regularity. With a few exceptions, which will be dealt with presently, the data suggest that wherever the rice-maize mixture contains over 0.35 per cent. P_2O_5 no deficiency is manifested. Between 0.35 per cent. and 0.30 per cent. the results are very irregular. In some cases the birds remained perfectly healthy, whilst in others they succumbed to polyneuritis after varying intervals. Thus at 0.34 per cent. P_2O_5 we have nine birds represented, of which two succumbed, one after sixty-nine days and one after ninety-eight days; the remaining seven were perfectly healthy, and their weight records gave no evidence of deficient feeding. When the diet is near the border-line of efficiency it is evident that the individuality of the birds is the dominating factor. One bird may remain perfectly healthy while its two cage fellows succumb, or vice versa. Thus in cage 12 two birds developed chronic polyneuritis, while the third remained perfectly healthy, without loss of weight. In cage 35 two succumbed and one remained healthy. The individuality factor is still more clearly brought out in cage 7, where bird 19 developed polyneuritis, although its two cage fellows, together with all three birds in cage 6 on a *lower proportion of maize*, did not. Except in the case of this bird of obviously higher vitamine requirements than usual, and cage 25 on special table meal, no case of polyneuritis occurs on a mixture containing *over* 0.35 per cent. P_2O_5 . Between 0.35 per cent. and 0.38 per cent. we have thirty-three birds represented, of which thirty remained healthy and in excellent condition until put out of experiment after four months' feeding. And it does not seem to matter whether the mixture is made up with rice mixed with whole meal, milled meal, bran, or germ meal. As soon as the P_2O_5 content is brought to about 0.38 per cent. the mixture becomes efficient.⁶ This suggests that the phosphoric acid of maize *can* be taken as guide to the vitamine content, and superficially (ignoring cage 25) one might be tempted to accept it as such. Strictly, however, this is not justified, since all the maize products used have been derived from a few different samples of whole grain, and, where the number of origins is small, chance may easily ordain that they be similar. There are also a few disconcerting cages which point in the

⁶ In this connection the indicator limit of 0.4 per cent. P_2O_5 for rice, suggested by Frazer and Stanton, may be recalled. "Etiology of Beri-beri." Studies from the Institute of Medical Research, Federated Malay States, No. 12, 1911.

opposite direction, and suggest that the connection between phosphoric acid content and vitamine content is purely arbitrary. Comparing cages 41 and 42 with cage 25 we have all three birds going down on the special meal "J" within the short period of nineteen days in spite of its 0.38 per cent. P_2O_5 content, while the whole grain "Q" can stand dilution to 0.31 per cent. P_2O_5 (with twice its weight of polished rice) and still remain efficient for all three birds. Diluted down to 0.28 per cent. P_2O_5 it becomes comparable with "J," although the survival periods of the pigeons concerned are still much longer. It is hardly likely that the difference could be explained on the individual idiosyncrasy of the birds—that in cage 25 we happened to have struck three particularly susceptible birds, and in cage 42 three particularly resistant birds. Such a supposition would strain the legitimate probabilities involved in so limited a test.

The explanation which fits these cages, as well as accounting for the general indicator limit 0.35 per cent. to 0.38 per cent. P_2O_5 , suggested by the other cages, is that *phosphoric oxide is only an indication of the distribution of vitamine in individual grains, but not an indication of the vitamine present in different grains of different phosphoric oxide content.* Cage 25 would then represent a sample of maize of originally high P_2O_5 content, highly milled and so devitaminized but still containing a high amount of residual P_2O_5 in spite of removal of its pericarpal layers. Cages 41 and 42 would then correspond to a natural meal of comparatively low P_2O_5 content, but of normal vitamine content.

We may assume that there is no relationship between the natural P_2O_5 content of the whole grain and the natural vitamine content; or we may still assume that a high P_2O_5 content is associated with a high vitamine content, but that the two do not run strictly parallel.

Absence of Correlation between Vitamine and Phosphorus in Different Samples of Maize.

To test this point it was necessary to secure as wide a range of whole mealies as possible. About twenty samples were purchased at random from different sources without reference to breed, and after analysis arranged in order of P_2O_5 content. Fortunately the range was fairly wide, the lowest sample showing 0.35 per cent. and the highest 0.71 per cent. Intermediate samples were then picked out so as to offer a rising increment of approximately one-fifth between successive samples, and each was tested in four dilutions with low P_2O_5 (0.1 per cent.) samp as deficient base. Samp was used in preference to polished rice in order to keep the diets restricted to the maize grain. The samples of whole grain, two of which were yellow mealies (No. 2 being "Chester County") and four white mealies (No. 5 being "Hickory King"), were ground, mixed with the ground samp in the percentage proportions indicated (Table II), and hand-stuffed as usual in quantities corresponding to one-twentieth of the body-weight of the cages concerned. The test involved the use of seventy-two birds, and the results are given in the following Table II. A blank in the last column denotes healthy survival without loss of condition for four months, and therefore indicates vitamine adequacy of the mixture concerned. The survival expressed in days denotes that polyneuritis or death occurred after feeding for the period indicated.

TABLE II.

Showing the absence of connection between P_2O_5 and Vitamine in
DIFFERENT samples of whole maize.

Cage No.	Sample No. of Maize.	Percentage P_2O_5 in the Maize.	Percentage of		Percentage P_2O_5 in the Mixture.	Survival of Pigeons in Days.		
			Grain in the Diet.	Samp in the Diet.		a.	b.	c.
51	1	.35	30	70	.175	21	26	29
52			50	50	.225	—	—	—
53			60	40	.250	—	50	—
54			70	30	.275	—	—	—
55	2	.42	30	70	.196	18	28	28
56			50	50	.260	24	60	25
57			60	40	.292	—	40	—
58			70	30	.324	—	—	—
59	3	.49	30	70	.217	24	41	32
60			50	50	.295	41	21	47
61			60	40	.334	—	—	—
62			70	30	.373	—	—	—
63	4	.57	30	70	.241	70	17	100
64			50	50	.335	—	—	—
65			60	40	.382	—	—	—
66			70	30	.429	—	—	—
67	5	.62	30	70	.256	38	25	27
68			50	50	.360	42	—	—
69			60	40	.412	—	82	—
70			70	30	.464	—	—	—
71	6	.71	30	70	.283	45	28	34
72			50	50	.405	—	48	—
73			60	40	.466	—	72	—
74			70	30	.527	—	—	—

The data are striking, and according to our own previous conceptions and the widely held assumption underlying the advocacy of phosphoric oxide as indicator of vitamine, somewhat unexpected. There is no doubt at all that the P_2O_5 content bears very little relation to the antineuritic efficiency. Broadly considered, the vitamine content may be regarded as the same in all six samples in spite of the fact that the P_2O_5 content from lowest to highest differs by over 100 per cent.—0.35 per cent *versus* 0.71. In every case a mixture containing 70 per cent. of samp and only 30 per cent. of whole grain is shown as deficient, the survival periods ranging from twenty-one to forty-five days, and the P_2O_5 content of the mixture from 0.175 per cent. to 0.283 per cent. In every case also a mixture containing 30 per cent. of samp and 70 per cent. of whole grain is shown to contain sufficient vitamine for pigeon requirements. For the mixtures containing samp and maize in equal proportions, deficiency is indicated by eight birds out of eighteen, and this proportion is therefore suggested as being on the border-line of deficiency. With the mixtures containing 60 per

cent. of maize and 40 per cent. of samp, fourteen out of eighteen birds remained perfectly healthy, while four, apparently of somewhat higher requirements than usual, succumbed. This mixture may therefore be taken as containing rather more than sufficient vitamine for average pigeon requirements.

The vitamine content of the mixture appears to depend wholly on the proportion of whole maize given and not on the phosphoric oxide content of the mixture, and no correlation between P_2O_5 and vitamine is discernable. Thus, with the sample of lowest phosphorus content, all three birds survived on a mixture containing as little as 0.225 per cent. P_2O_5 , while with the sample of highest phosphorus content all three succumbed on a mixture containing 0.283 per cent. P_2O_5 , and one succumbed on a diet as high as 0.466 per cent.

Whether a maize very rich in phosphoric acid does or does not contain a *little more* vitamine than one which is very poor in phosphoric acid, it is impossible to say from the data before us. It would appear *not*, but a test upon several hundred birds would be required to settle the point. Individual idiosyncrasy of the pigeons themselves dominates the situation where small numbers are concerned, and in work of this kind a large number of tests must frequently be carried out to settle a small controversial point. In the tests just quoted, for instance, if we consider samples 1 and 3, and look only at the mixtures of samp and grain in equal proportions (cages 52 and 60), we have, for the first, healthy survival of all three birds, and for the third three clear cases of polyneuritis within fifty days. Drawing conclusions from these six birds *alone*, it would seem quite clear that the first mixture is efficient, and the second deficient in spite of the fact that it contains as much whole meal and 40 per cent. more P_2O_5 ; we would be inclined to put down sample 1 as richer in vitamine than sample 3. But if we consider the same two samples *only* in the mixtures containing 60 per cent. of maize to 40 per cent. of samp, the position is reversed, and sample 3 is suggested as richer in vitamine than sample 1. Surveying the whole seventy-two birds together, however, it is quite apparent that the differences noted between the samples are attributable to variation in the individual vitamine requirements of the different pigeons. This may be brought out more clearly if we arrange the data of Table 2 in relative numbers with 100 as base. For vitamine content we may fix *the vitamine requirements of the average pigeon* as base (100), and from this reference number draw up percentage indices showing the proportion of vitamine in the other mixtures. To do this, however, we have to select relative values for samp and maize. On the view already expressed (as suggested by the data of Table I) that the distribution of vitamine in the individual grains follows the distribution of phosphorus, we may provisionally assume that the residual vitamine of samp corresponds to its residual phosphorus and is therefore approximately one-fifth of that present in the whole grain from which it was derived.

As already pointed out, a mixture of maize: samp: : 50: 50 appears to be on the border-line of efficiency, since eight out of eighteen birds succumbed on it, while a mixture of maize: samp: : 60: 40 appears to contain vitamine slightly above average requirements, since fourteen out of eighteen birds remained perfectly healthy upon it. An intermediate mixture probably represents the mean requirements of the birds concerned, and we may therefore take maize: samp: : 55: 45

as representing an adequate average mixture as closely as the data allow us to gauge it. Assigning the basal vitamine index 100 to this mixture, whole maize grain takes the index 156 and samp 31. The vitamine indices for the mixtures 30: 70, 50: 50, 60: 40, 70: 30 then work out at 69, 94, 106, 118. In Table III all six samples of maize are treated as containing the same amount of vitamine, so as to bring out the comparison with the varying P_2O_5 content. The P_2O_5 indices are referred to maize of *average* phosphorus content, Vipond's⁷ average of .545 per cent. P_2O_5 for twenty-three *South African grown* samples being taken. The efficient minimum maize: samp: : 55: 45 would thus average .345 per cent. P_2O_5 . Equivalating this to 100 the P_2O_5 indices then show the variation in the mixtures according to the variation in the different samples examined. For sample 4 the vitamine indices and P_2O_5 indices correspond approximately, since this sample is not far from average in phosphorus content. For the samples of highest and lowest P_2O_5 the divergence between the two indices is very apparent.

TABLE III.

Bringing out the comparison between vitamine content and phosphorus content of different samples of whole maize and specifically illustrating the absence of connection by reference to South African maize of average P_2O_5 content (.545 per cent.) and mixtures containing vitamine in amount adequate for the average pigeon.

In the mixtures .345 per cent. P_2O_5 = index 100. Vitamine index 100 = average pigeon requirements.

Cage No.	Maize Sample No.	Per cent. P_2O_5 .	Diet Mixtures.				Character of the Mixtures as Indicated by the Fate of the Pigeons.		
			Grain.	Samp.	P_2O_5 Index.	Vitamine Index.	a.	b.	c.
51	1	.35	30	70	51	69	deficient	deficient	deficient
52			50	50	65	94	adequate	adequate	adequate
53			60	40	72	106	"	deficient	"
54			70	30	80	118	"	adequate	"
55	2	.42	30	70	57	69	deficient	deficient	deficient
56			50	50	75	94	"	"	"
57			60	40	85	106	adequate	"	adequate
58			70	30	94	118	"	adequate	"
59	3	.49	30	70	63	69	deficient	deficient	deficient
60			50	50	86	94	"	"	"
61			60	40	97	106	adequate	adequate	adequate
62			70	30	108	118	"	"	"
63	4	.57	30	70	70	69	deficient	deficient	deficient
64			50	50	97	94	adequate	adequate	adequate
65			60	40	111	106	"	"	"
66			70	30	124	118	"	"	"

⁷ Analyses by H. J. Vipond, quoted in "Maize," p. 666. J. Burt-Davy. Longman & Co.

TABLE III—(continued).

Cage No.	Maize Sample No.	Per cent. P_2O_5 .	Diet Mixtures.				Character of the Mixtures as Indicated by the Fate of the Pigeons.		
			Grain.	Samp.	P_2O_5 Index.	Vita- mine Index.	a.	b.	c.
67	5	62	30	70	74	69	deficient	deficient	deficient
68			50	50	104	94	"	adequate	adequate
69			60	40	119	106	adequate	deficient	"
70			70	30	134	118	"	adequate	"
71	6	71	30	70	82	69	deficient	deficient	deficient
72			50	50	117	94	adequate	"	adequate
73			60	40	135	106	"	"	"
74			70	30	152	118	"	adequate	"

Examination of the data in the form presented in Table III at once justifies the assumption that all six samples of maize contain approximately the same amount of vitamine in spite of their widely varying P_2O_5 content. In all cases the pigeons register the diet as deficient for vitamine index 69, i.e. where the amount of vitamine is 31 per cent. below average requirements. The diet is then so deficient that every bird tested succumbs. In all cases vitamine index 118 is registered as adequate by the pigeons, i.e. 18 per cent. above average requirements has supplied enough vitamine for every bird tested.

Over the range 94-106, i.e. 6 per cent. above or below average requirements, we would expect biological variations to be prominent, and this is exactly what we find. Individual idiosyncrasy here dominates the results. On the mixtures "maize: samp: : 50: 50" all three birds register efficiency in the cases of samples 1 and 4, all three register deficiency with samples 2 and 3, while for samples 5 and 6 one bird registers deficiency, and two register efficiency. The order of results is not in order of P_2O_5 content, but simply suggests that the mixtures concerned are so close to the border-line of efficiency that the appearance of polyneuritis is erratic. The value "94 per cent. of average requirements" is therefore justified for all six cages. For cages Nos. 53, 57, 61, 65, 69, and 73 on the mixtures "maize: samp: : 60: 40" the data again fail to show any definite order of efficiency for the different samples. The diet derived from samples 1, 2, 5, and 6 all claimed one victim out of three, while samples 3 and 4 did not. Again, the results indicate that the mixtures are near the border-line of efficiency, and the value 106 for all six cages is probably as near the truth as we can get. Taking the 36 birds on the border-line range, vitamine index 94-106, ten may be taken as sub-normal in requirements to the extent of at least 6 per cent. Four are above normal to the extent of at least 6 per cent. The remaining twenty-two do not necessarily vary from the theoretical "average pigeon" by more than ± 6 per cent. If the range of mixtures had been more extended so as to vary by a greater number of indices evenly spaced around the average, it is *not improbable* that all thirty-six

birds would have suffered by vitamine index 90 and survived on vitamine index 110. At any rate, in view of the irregular character of the variation actually shown it seems perfectly legitimate to conclude that the six different samples of maize do not vary more than ± 10 per cent. in their vitamine content, i.e. that the difference between highest and lowest is not more than 20 per cent., the probable range of individual idiosyncrasy of the pigeons used to test them. They probably vary less, and in any case there is no evidence to show that the vitamine content and the phosphorus content are in any way correlated. On the contrary all the evidence goes to show the absence of correlation. The pigeons make no distinction between any of the samples. They respond to sample 1 with .35 per cent. P_2O_5 in the same way as they do to sample 6 with .71 per cent. P_2O_5 ; indeed the idiosyncrasy of the individual happens to cast against the higher sample—for the border-line mixtures two birds succumb out of six on sample 6 and only one on sample 1. According to the P_2O_5 indices every cage on sample 1 should be markedly deficient, and cages 72 and 73 on sample 6 should be adequate for all the birds concerned.

If P_2O_5 could be taken as "indicator of vitamine" cages 54 and 71 should behave in the same way. Yet the former is thoroughly adequate and the latter markedly deficient for all three birds in each case. One would expect *every* bird in cage No. 54 to succumb (P_2O_5 index 80) as well as in cages Nos. 53 and 52 (P_2O_5 indices 72 and 65), whereas one would anticipate survival for bird "b," cage 72 (P_2O_5 index 117) and most certainly expect it with bird "b," cage 73 (P_2O_5 index 135). Judged by the phosphorus standard, sample 6 should contain twice as much vitamine as sample 1. Such a difference could not have escaped detection, yet the pigeons do not differentiate between the two at all.

REAL CORRELATION BETWEEN DISTRIBUTION OF VITAMINE AND DISTRIBUTION OF PHOSPHORUS IN INDIVIDUAL GRAINS.

As already remarked, the data of Table I indicate a parallelism between phosphorus content and vitamine content in maize and its milling products. Whether the polished rice mixtures were made up with whole maize, milled meal, bran or hominy chop, vitamine adequacy was reached as soon as the P_2O_5 content reached 0.33 per cent. to 0.38 per cent. But it has just been shown that in different samples of maize phosphorus is an independent variable fluctuating between wide limits without noticeable variation in vitamine content. The deduction therefore is that the correlation between vitamine and phosphorus only affects the distribution in the kernel itself.

To test this point a little more specifically than was done in the tests of Table I, a further series of trials was put up. The commoner milling products, all stated to be derived from the same load of grain, were again put up in varying dilutions, but this time using samp as deficient base, in order to maintain the tests within one sample of material. The mixtures were made up in proportions (Table IV) calculated from their P_2O_5 content in such a way that each set on one product was comparable with each set on another product, in respect to phosphorus. The mixture of maize : samp : : 55 : 45, gauged as representing the vitamine requirements of the average pigeon, was taken as basis for comparison. The P_2O_5 content of this mixture works out

at .378 per cent. Mixtures of phosphorus content 10 per cent. below (.341) and 10 per cent. above (.416) this figure were then worked out, this range being calculated to cover the border-line of biological variation of the test-birds. The mixtures for all the products examined, calculated as equivalent in P_2O_5 to those of the whole maize set, are given in Table IV. The mixtures were usually made up in quantities of one or two kilos at a time, e.g. for cage No. 82, 487 gm. of seconds would be mixed with 513 gm. of ground samp, reground to ensure homogeneity, and bottled. Mode of feeding, preparation, and quantity of food were the same as in the preceding tests.

The column headed " P_2O_5 Index" expresses phosphorus relatively to .378 as 100, and catches the eye better than odd decimal figures. If the distribution of vitamine and of phosphorus run parallel, this column should also read "vitamine index." If the behaviour of the pigeons justifies this reading then we have strong evidence that the location of vitamine in the kernel follows the location of phosphorus.

Table IV represents the experimental results on forty-eight birds.

TABLE IV.

Showing the real correlation existing between distribution of vitamine and distribution of phosphorus in the kernel of maize when a single sample of grain is considered. In the last column a blank indicates healthy survival for at least 120 days.

Cage No.	Material.		Diet Mixtures.				Survival of Pigeons in Days.		
	Kind.	Percentage P_2O_5 .	Material.	Samp.	Percentage P_2O_5 .	P_2O_5 Index.	a.	b.	c.
75 } 76 } 77 }	Maize	0.59 {	47.0	53.0	0.341	90	52	61	—
			55.0	45.0	0.378	100	60	—	49
			63.0	37.0	0.416	110	—	71	—
78 } 79 } 80 }	Fine meal	0.46 {	65.0	35.0	0.341	90	39	53	65
			76.0	24.0	0.378	100	—	50	42
			87.0	13.0	0.416	110	—	—	—
81 } 82 } 83 }	Seconds	0.65 {	41.7	58.3	0.341	90	45	—	51
			48.7	51.3	0.378	100	—	—	—
			56.0	44.0	0.416	110	—	—	—
84 } 85 } 86 }	Bran	0.67 {	40.2	59.8	0.341	90	41	—	—
			47.0	53.0	0.378	100	—	—	—
			53.8	46.2	0.416	110	—	—	—
87 } 88 } 89 }	Hominy chop*	1.42 {	17.0	83.0	0.341	90	38	53	71
			19.8	80.2	0.378	100	—	45	—
			22.8	77.2	0.416	110	—	—	—
90	Samp	0.42	Deficient Base of Mixtures				9	11	14

* NOTE.—Hominy chop contains the germ as well as the outer layers of the grain. Unfortunately isolated germ was not procurable in a reasonably pure state as a separate milling product.

Surveying this table as a whole, there is not much doubt that, in so far as can be deduced from the examination of milling products, the distribution of vitamine and phosphorus in the kernel correspond quantitatively within the limits of detection by a moderate number of pigeons.

In every case except one, i.e. in fourteen out of fifteen cases, .416 per cent. P_2O_5 is shown to correspond to vitamine adequacy. This fits very well with the assumption that P_2O_5 index 110 represents 10 per cent. more vitamine than the normal pigeon requires, and therefore suggests a quantitative parallelism between phosphorus and vitamine in the kernel. The exception, bird No 77b, merely means that this pigeon happens to vary by more than 10 per cent. above the normal, just as bird No. 75c varies by not less than 10 per cent. below. The survival of two out of three birds in cage 77, and the collapse of two out of three in cages 76 and 75, is all evidence of individual variation on the border-line of dietary deficiency. For these three cages the vitamine is obviously in the proportion of about 90 : 100 : 110, since the same two components are used in compounding all three mixtures. Individual variation in response to vitamine supply therefore exceeds the ± 10 per cent. assessed as probable in discussing Table III. We are not in the position to judge of possible extremes of biological variation, but if an occasional pigeon showed a very marked deviation from his fellows it should occasion no surprise. For mixtures of P_2O_5 content .341 per cent., index 90, two birds succumb on maize, three on fine meal, two on seconds, one on bran, and three on hominy chop. This corresponds quite well to probable individual variation on border-line mixtures containing 10 per cent. less vitamine than the average pigeon requires. Phosphorus is therefore again acceptable as indicator of vitamine.

For P_2O_5 .378 per cent., index 100, we have a survival of ten birds out of fifteen, a proportion quite natural on the assumption that all the mixtures contain just enough vitamine for average demands, but not enough for individuals of slightly higher requirements.

It is true that bran is suggested as containing rather more vitamine in proportion to phosphorus than the other products. Out of nine birds in the test only one succumbs, as against five for the whole maize mixtures and four for hominy chop, but it is difficult to interpret this fact accurately. The supposition that the six birds in cages 85 and 86, which survive at index 100 or over, and the one victim of cage 84, which naturally succumbed on 10 per cent. less than average requirements, are *normal*, and that the two survivors of cage 84 are subnormal like bird No. 75c, fits the facts. But the facts are equally well fitted by supposing that this one victim is also normal, and that the other eight birds all survive because the vitamine content of the bran is really a little higher than that indicated by its phosphorus content. There is another possibility which appeals to us as the most probable, and that is that the phosphorus and vitamine do correspond, but that the mixtures containing a large proportion of bran have a lower digestible value than the mixtures in which samp or maize predominate, and that in consequence the real proportion of absorbed vitamine to digested metabolized food is higher. The thesis that the requirements of vitamine in metabolism are met, not in terms of a fixed intake per bird, but in terms of the gross energy metabolism,

is being developed in a later paper and need not therefore be elaborated here. For the moment the explanation of the slight discrepancies of the bran tests may be left in abeyance. The results already given show that in so far as a limited examination of milling products is concerned the distribution of phosphorus and of vitamine in the kernel may be regarded as coinciding to within a probable error of ± 10 per cent. It is quite likely that the parallelism is even closer, but to determine this would involve application of the statistical method on a large number of birds so as to rule out idiosyncrasy, and the examination of a greater range of separate products obtained by milling in different ways, or by isolating some portions, such as the germ, by dissection.

Of course the parallelism in location of phosphorus and vitamine implies no necessary chemical or physiological connection. It does seem probable, however, that the connection is not altogether arbitrary, and that, although the functions of neither are properly understood, they are definitely correlated physiologically in the cell metabolism of the plant.

Before leaving the data of Table IV it is worth commenting upon the comparison it offers with Table I in regard to the amount of *residual vitamine* present in samp and in polished rice respectively. Considerably more vitamine-rich supplement is required to meet the deficiency of samp than to meet the deficiency of polished rice, and the conclusion is therefore that the latter contains considerably more residual vitamine than the former. If we compare cages 5, 6, and 7 of Table I with cages 75, 76, and 77 of Table IV we have:—

Cages with Rice	Survival in Days.			Cages with Samp.	Survival in Days.		
5	50	80	105	75	52	61	—
6	—	—	—	76	60	—	49
7	80	—	—	77	—	71	—

The general run of results indicates that cages 6 and 76 both contain approximately the adequate amount of vitamine for the average pigeon, since both are on the border-line of efficiency, and in each case cages of vitamine content a little below and a little above claim victims. For tentative comparison we may therefore equvalate them. The diet of the three birds for cage 6 was made up by mixing 15 grm. of maize with 28 grm. of rice, i.e. in the approximate proportions maize: rice: : 35: 65, as against maize: samp: : 55: 45 for cage 76. These proportions would indicate that rice contains approximately twice as much residual vitamine as samp. Using the provisional vitamine indices adopted for calculating Table III, i.e. accepting samp as 31 and maize as 156, the value of polished rice actually works out at 69. The suggestion therefore is that polished rice, always considered a highly deficient diet, and often treated as if it were vitamine-free, may in reality contain approximately two-thirds of avian or human average requirements.

EFFECTS OF COOKING MAIZE UPON ITS ANTINEURITIC EFFICIENCY.

A few tests were also carried out to determine the thermal stability of the antineuritic vitamine in maize. The effect of autoclaving at different temperatures was tested; also the effect of various treatment suggested by the various modes of cooking in vogue.

(A)—Mealie pap, i.e. maize meal cooked by gently boiling in an open pot for two hours.

(B)—Maize meal left steeping in water for twenty-four hours and then boiled into pap.

(C)—Maize meal boiled into pap and then autoclaved for three hours at 118° to 122° C. This treatment was considered more severe than the method of cooking by super-heated steam in open boilers, as practised in the Rand mines' kitchens.

(D)—Maize meal mixed into a paste with water, and autoclaved in trays 2 inches deep for three hours at 123° to 126° C. There is no doubt that in such shallow cakes the heat permeated the whole mass.

(E)—Maize meal as in (D), but autoclaved for three hours at 130° to 133° C.

(F)—Maize meal roasted dry in layers about 1 cm. deep for three hours in a hot-air oven at 135° C.

The results are indicated in Table V. Blanks in the table denote healthy survival for at least four months, and therefore indicate vitamine adequacy of the birds concerned.

TABLE V.

	A.			B.			C.			D.			E.			F.		
Treatment of Maize Meal.	Boiled for Three Hours.			Steeped and Boiled.			Autoclaved for Three Hours at									Roasted at 135.°		
							118°-122.*			123°-126.°			130°-133.°					
	a.	b.	c.	a.	b.	c.	a.	b.	c.	a.	b.	c.	a.	b.	c.	a.	b.	c.
Survival of pigeons in days.....	—	—	—	—	—	—	—	—	—	—	—	—	12	19	24	21	14	25

It is quite apparent that fairly drastic treatment has to be applied before noticeable destruction of vitamine is effected. Autoclaving at 125° C. (fluctuating from 123° to 126°) was ineffective. At 130° C., however, destruction did occur; also on dry roasting at 135° . This thermo-stability of course only applies to the beri-beri vitamine, and not necessarily to any other class of vitamine which may possibly be present, but the presence or absence of which is not so easily demonstrated.

SUMMARY AND CONCLUSIONS.

(1) The examination of maize-milling products by dietetic experiments upon pigeons indicates that the distribution of vitamine in the

individual maize kernel follows the distribution of phosphoric oxide. Both are located chiefly in the sub-pericarpal layers and germ, to a less extent in the outermost skin, and to a very much less extent in the endosperm. In the sampling of maize about seven-eighths of the vitamine passes into the "hominy chop" (complete by-product), while about one-eighth probably remains in the "samp" (endosperm). Of the whole maize, about two-thirds is usually converted into samp and one-third into hominy chop, so that the latter is about twelve times as rich in vitamine as the former, or well over twice as rich as the original grain. If we take the *average* pigeon requirements as standard and state this as 100, we get a series of figures representing the amount of vitamine present as percentage of avian requirements. The probable error of the numbers is assessed as about ± 10 per cent.

Product.	Percentage P_2O_5 .	Vitamine in percentage of average requirements, ± 10 per cent.
Whole maize.....	0.59	156
Fine meal.....	0.46	122
Seconds.....	0.65	172
Bran.....	0.67	177
Hominy chop.....	1.42	375
Samp.....	0.12	31

These figures are relative and based upon the data acquired so far. The actual numbers depend upon the estimate of average requirements, and closer approximation would involve the use of statistical methods. The value for samp is interpolated, but is probably fairly near the truth. The vitamine values of milling products of course vary with the mode of milling, and the grade of "fine meal" arrived at. "Bran," for example, would be expected to run higher in general than in the case just cited, where the material appears to consist chiefly of the outermost skin. It seems probable that an average value for millers' mealie bran, containing a higher proportion of the sub-pericarpal layers and germ and a larger fraction of the total phosphoric oxide of the grain, would run nearer 300 than the value given here. It depends upon what fractions happen to be slumped as "bran."

(2) Milled meals and table preparations vary in vitamine content according to the extent of milling, and may range from highly deficient products (fanko, samp, and "specially prepared table meals") with about one-third of the vitamine actually required for healthy maintenance, up to efficient products containing 20 per cent. to 40 per cent. more vitamine than is actually demanded by the average individual. Meals milled for native consumption and the ordinary meals of commerce appear to be quite efficient, but high-grade table meals and flours are usually deficient.

Whole maize can stand depletion of about one-third of its phosphoric oxide in the process of milling before becoming deficient for the *average* pigeon, or presumably the average man. When supplementary rations comparatively rich in vitamine are used, a meal milled to this extent would be safe; but where little or no supplement is

eaten, such a border-line grade would probably contain too little vitamine for those individuals of higher requirements than usual, and a not inconsiderable incidence of deficiency diseases might be expected. If the supplement is meagre it would therefore be safer to mill more lightly. Reduction of the percentage of phosphoric oxide by about one-sixth in the process of milling would probably still leave the meal efficient for all individuals in so far as beri-beri vitamine is concerned, and at the same time considerably reduce the crude flavour and coarse texture objected to in whole meal. This would still leave the meal with a vitamine index 130 or 30 per cent. above *average* requirements. If we accept the common medium grade fine meals and flours as containing four-fifths of the percentage of P_2O_5 in the original grain, they would have a vitamine index of 120 to 125, or 20 per cent. above average requirements. Such fine meals should therefore never come under suspicion as staple of a *mixed ration*, since a very small supplement richer in vitamine should throw the diet above the danger zone for even those rare individuals of exceptionally high vitamine requirements who might conceivably be hit by the fine meal alone.

(3) The parallelism between phosphoric oxide and vitamine content *does not hold between different samples of maize*, but only for distribution in any given kernel.

In a series of six different samples varying in P_2O_5 content by over 100 per cent. no difference in vitamine content could be detected by pigeon analysis:—

	1.	2.	3.	4.	5.	6.
Percentage P_2O_5 in different samples of whole maize.....	.35	.42	.49	.57	.62	.71
Amount of vitamine present	All six indicated as containing from 50 to 70 per cent. above average requirements for health.					

In these samples the "indicator limit" of phosphoric oxide for border-line milled meals would range from about 0.23 per cent. to 0.46 per cent. It is therefore impossible to use phosphoric oxide as indicator of vitamine unless the phosphoric oxide content of the original mother grain be known. This information is rarely available, and the determination of P_2O_5 as a general analytical guide to efficiency—as advocated by Voegtlin, Sullivan, and Myers⁵—is therefore ruled out of court. Their standard for maize flour, which assumes that "*the P_2O_5 content should not be less than 0.5 per cent.*" would condemn more safe samples than it passed. It would correspond to samples on the border-line of efficiency if the mother grain from which they were derived contained the relatively high proportion of 0.78 per cent. P_2O_5 , and all ordinary meals coming up to their standard would certainly be efficient. But it would lead to false conclusions about all meals derived from mother grain containing less than 0.6 per cent. P_2O_5 , and these predominate, especially in South Africa. Such a standard therefore is perfectly safe, but has no useful application.

⁵ U.S. Health Reports, 14th April, 1916. Ref. Tropical Diseases Bulletin, Vol. VII, No. 7.

The much lower indicator limit of 0.35 per cent. P_2O_5 would correspond to the border-line of efficiency for meals derived from *average* South African grain, and 0.4 per cent. P_2O_5 would probably ensure a safe margin of vitamine for average maize meals, but such a limit would reject just about as many healthy samples as it would pass deficient ones. The only sound judgment would be one based upon the extent of milling, and in absence of information about the original grain microscopic examination would provide a safer criterion than P_2O_5 estimation. The biological test is of course the rational one but this, involving a graduated series of tests upon pigeons, is laborious and lengthy, and not within the scope of the usual analytical laboratory.

It is morally certain that the same line of reasoning is applicable to rice and to other cereals. Frazer and Stanton's indicator limit of 0.4 per cent. P_2O_5 for rice is now largely applied in the Far East, a sample containing less than this being regarded as "polished." In the Phillipines it has been used as a guide to legislative control of the extent of polishing. No doubt it deals fairly well with *average* samples of rice originally containing 0.5 to 0.6 per cent. P_2O_5 , the average from which it was deduced by Frazer and Stanton being 0.54 per cent. (l.c. 6, p. 56), but it would almost certainly condemn vitamine-adequate lightly polished samples derived from rices grown under conditions of soil and climate which tended to produce a grain of low natural P_2O_5 content. Occasionally it might pass a sample just below the border-line of efficiency where the original grain had been above the average in phosphoric oxide, but since the diet of even the poorest labourer nowadays is to some extent "mixed" the supplementary moiety would generally cover a *slight* deficiency, and any analytical error of judgment would escape detection. On the other hand where safe samples were condemned as being "too low in phosphoric oxide" the grower or vendor would know no better and not be in the position to protest.

The fact that the standard of Frazer and Stanton has proved useful in practice is no argument that it (or any other P_2O_5 standard for any other meal) is universally applicable, and it is worth bearing in mind that these two authors put it forward with due scientific caution in reference to their own experience in the Malay States.

(4) Maize meal as such can stand autoclaving at 125° C. for several hours without noticeable destruction of vitamine, although at 130° C. or over destruction is rapid. At 125° C. the food is rendered somewhat unpalatable. It is highly improbable that any of the methods in vogue for the cooking of maize are likely to engender deficiency in respect to beri-beri vitamine.

(5) Highly polished rice is shown to contain considerably more vitamine than samp, and is suggested as still containing at least half, and perhaps two-thirds, of average avian or human requirements. This would explain why it is that a small supplementary ration of moderate vitamine content added to a rice diet has often been found to protect from beri-beri.

(6) The incidence of scurvy on the Rand mines, statistically always low (Orenstein), has probably nothing to do with the vogue of milling of the maize for native consumption, but is more likely to be connected with the nature and quantity of the supplementary ration. So long as the moiety of fruit or vegetables is not cut down, scurvy does not seem to appear.

Upon the Quantitative Relationship between the Antineuritic Value of a Diet and the Onset of Polyneuritis.

BY

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From the Onderstepoort Laboratories for Veterinary Research.

Pretoria, November, 1917.

Upon the Quantitative Relationship between the Antineuritic Value of a Diet and the Onset of Polyneuritis.

By HENRY H. GREEN, D.Sc., Biochemist, Division of Veterinary Research, Onderstepoort.

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THE mass of literature dealing with the problems of health, maintenance, and growth upon limited or synthetic diets is now so voluminous, and comprehensive reviews of the subject are now so readily accessible, that it is needless to offer any introduction to a contribution dealing with any of the factors involved.

It is universally recognized¹ that, apart from the protein content and the calorific value of a diet, certain "minimal" substances must be present in order that the life-cycle of an organism may be completed. These need not *necessarily* contribute to the dynamic requirements or structural framework of the animal, but their presence in small amount in the food is essential for health and normal development. They are variously referred to by different authors, the terms "accessory food-stuffs" (Hopkins) and "vitamines" (Funk) being generally interchangeable in considering the higher animals. For accessory bodies essential to the development of lower organisms, such as yeasts, the term "bios" (Wildiers) has been used. In relation to bacterial growth the work of Twort and Ingram on Jöhnes bacillus, which in laboratory cultures will only grow on a medium to which dead tubercle bacilli or other acid-fast bacilli have been added, is of special interest. For accessory factors in plant nutrition Bottomley has used the term "auximones."

Little is known about the number, chemical constitution, mode of physiological action, or general inter-relationship of these accessories, but there is little doubt that, even when only one type of organism is considered, there may be several. Thus McCollum² and his co-workers, working chiefly on rats, have recently offered strong evidence to show that a distinction can be drawn between two unidentified dietary factors, "fat-soluble A," and "water-soluble B," both of which are essential for growth and maintenance.

It is with this "water-soluble B," "antineuritic hormone," or "beri-beri vitamine" that we are immediately concerned.

According to the conventionally accepted method of testing a foodstuff for the presence of this accessory, varying quantities of the material are added to a fixed basal ration of highly polished rice and fed to pigeons. The minimum quantity which has to be added in

¹ Röhmann may be regarded as the last of the old guard who still take exception to the "accessory foodstuffs" hypothesis, and believe that if the long familiar nutrients are suitable in quality and quantity nothing further is essential in the ration. Osborne and Mendel have recently (*J. Biol. Chem.*, Vol. XXXI, No. 1, July, 1917) made a special point of dealing with Röhmann, disposing of his case in a fashion which will satisfy most other workers.

² *J. Biol. Chem.*, XXIII, 1915, XXV, 1916, and onwards; a series of papers in collaboration with Davis, Simmonds, Pitz, Hart, and Miller.

order to protect against the onset of "polyneuritis gallinarum" or "avian beri-beri," for a convenient arbitrarily selected experimental period, is thus determined. The pigeon is probably the most suitable experimental subject, since it responds rapidly to deficiency in the diet, is easy to feed quantitatively, and usually (not always) shows deficiency symptoms in a clear-cut unmistakable clinical fashion. Polished rice is notably deficient in antineuritic vitamine, and has hitherto been largely used as basal ration. Thus in Cooper's³ early tests 20 grm. of fresh meat or 3 grm. of lentils added to 15 grm. of highly polished rice (one-twentieth of the average pigeon body-weight) sufficed to protect against polyneuritis for a period of at least fifty days. Weight for weight, therefore, the content of lentils in antineuritic hormone is much higher than that of fresh meat.

This simple method suffices for most purposes and was adopted by us in determining the antineuritic value of certain rations fed to cattle in connection with experiments on the disease *Lamziekte*,⁴ which had been suggested as an avitaminosis, and erroneously compared with beri-beri in the human subject. But in attempting to assess the vitamine values of foodstuffs over a wide range, varying from bulky fodder such as grass to concentrated material such as beans, a number of questions of considerable theoretical and practical interest are raised. What are the limits of error in such biological analysis? How many pigeons represent a fair test and of what duration must the feeding trial be? What is the relationship between the amount of vitamine in a diet and the period elapsing before the onset of deficiency symptoms? Can a series of tests on varying inadequate quantities of vitamine-rich supplement be related to one another? What effect has the general composition of a diet, and the quantity fed, upon the vitamine requirements? In how far is the demand of an animal for vitamine dependent upon the diet and in how far upon the animal itself? Is a fixed absolute daily physiological minimum required for maintenance, or must the vitamine form a fixed percentage proportion of the diet? How do different classes of animals behave in their demand for vitamine? What is the best basis of comparison of different demands? If, for example, a pigeon upon a given diet develops polyneuritis in two months is it possible to estimate how long a man is likely to take to develop beri-beri upon the same diet?

The questions which might be raised are numerous and obvious, and indeed some of those mentioned have been frequently raised in the voluminous literature which centres round the beri-beri problem, although usually only in general fashion. Thus it has been maintained by Funk⁵ and others that pigeons develop deficiency symptoms more rapidly upon an ample diet than upon a meagre one, and some of our own results (*l.c.* 4) clearly illustrate this. But the fact that this view is by no means accepted, or at least by no means taken into calculation, by the generality of workers, is evidenced repeatedly in the current literature—usually by implication rather than by specific statement. As a recent example (1917) we may quote a passage from Osborne and Mendel's paper on "The Rôle of Vitamines in the Diet" (*l.c.* 1), in which, writing of a synthetic diet suspected to have still

³ *Journal of Hygiene*, Vol. XXII, p. 436, 1912.

⁴ Theiler, Green, and Viljoen. Contribution to the study of Deficiency Disease, with special reference to the *Lamziekte* Problem in South Africa. Third and Fourth Reports of the Director of Veterinary Research, Department of Agriculture, Union of South Africa.

⁵ *Proc. Physiol. Soc.*, Dec. 13, 1913, XXV.

contained a trace of vitamine (water-soluble "B") he says (p. 152) that "*it would seem as if such a diet were adequate for maintenance, provided that an animal can be induced to consume enough of it.*" We read this as implying that in eating a greater gross quantity of the food a larger absolute intake of vitamine would be assured, so sufficing for maintenance. This would then involve the assumption that the physiological demands of any given animal are more or less absolute and independent of the gross metabolism. All our own work on pigeons goes to negative this idea and to establish the contention that the larger the quantity of food eaten the more vitamine is required to metabolize it; if a diet is vitamine-poor, then the more of it consumed by the animal the faster do deficiency symptoms set in. We prefer to think of the rare cases of maintenance on diets of very low vitamine content as due to individual idiosyncrasy—very low demands on the part of the exceptional subject concerned.

Pigeons starved to death rarely show polyneuritic symptoms,⁶ thus indicating that the tissues contain at least sufficient vitamine to last out their own catabolism, though affording no indication of the actual dimensions of body vitamine reserves.

It has also been suggested that the anti-neuritic hormone is directly associated with carbohydrate metabolism, and that a starchy diet is more likely to give rise to beri-beri than a diet containing much protein and fat would do. There is, however, little evidence for this, and we have already (*l.c.* 4, p. 23) maintained that it is probably the gross energy metabolism rather than the specific carbohydrate metabolism which is the dominant factor. It is possible to produce polyneuritis on a diet consisting of fat and washed autoclaved mince-meat in as short a time as on polished rice.

But although there has been much speculation as to the quantitative vitamine demands of various animals on various diets there seems to have been no attempt to reduce the matter to any very definite numerical basis. A number of current "probable misconceptions" either arise from, or are responsible for, this neglect of numerical expression. One of these is an implicit treatment of polished rice as if it constituted a "vitamine-free" diet instead of merely a "deficient diet." Because nobody seems to have tried to indicate how much vitamine is still present, the majority of workers (not all) appear to proceed on the assumption that it contains none at all—or at most only negligible "traces." As will be contended presently, our own evidence suggests that it may contain as much as, or more than, half of total human or avian requirements.

The purpose of the present paper is to attempt a provisional working formula relating three of the factors involved in dietetic deficiency of the type under consideration—amount of vitamine in the food, quantity of the food, and survival time. Such an expression, whatever its limitations may subsequently be shown to be, at least provides a basis for numerical discussion, and a mode of co-ordinating many of our own results and some of the data occurring in the literature. It is too simple to be rigorous, and leaves out too many unknown factors to be generally valid, but it at least *attempts* to get at numbers

⁶ Eykman (*Geneesk. Tijdschr. v. Nederl-Indië*, 1916, Vol. LVI, No. 3, abstracted *Trop. Dis. Bull.*, Vol. VIII, No. 7), has shown that if *large quantities* of water are given typical polyneuritis may be induced within the period of "death by starvation." This is presumably a "washing out" effect. We have failed to produce symptoms in starved birds left to take water *ad lib.*

for quantitative comparison of one experiment with another, for disentangling the possible factors in apparently discordant duplicate tests, and for measuring the extent of idiosyncrasy in different subjects. It only professes to be tentative, and can be expanded or abandoned as future results ordain. Meanwhile we can see where it leads us.

In carrying out experiments to determine the anti-neuritic value of foodstuffs we were forcibly struck by what at first sight appeared to be an "all or nothing principle" dominating results, i.e. it appeared as if the "adequate" quantity of vitamine-rich foodstuff which had to be added to a deficient basal diet in order to render it efficient was sharply defined (*l.c.* 4, p. 21). As an illustration of this we may take a typical selection of our own earlier experimental results (Table I). Each test consisted of a cage of three pigeons, hand-fed with one-twentieth of the average bodyweight to ensure uniform intake. As will be observed, the deficient basal ration is reduced as the quantity of vitamine-rich supplement is increased, so as to keep the amount of food approximately constant.

TABLE I.

Pigeons hand-fed with mixtures equivalent to 1-20th of the average bodyweight of the three birds in the cage.

Cage.	Bird.	Diet per Bird.	Survival in Days.	Cage.	Bird.	Diet per Bird.	Survival in Days.
1	{ 1 2 3	{ 1 grm. of polished rice alone }	{ 17 25 29	9	{ 25 26 27	{ 15 grm. of polished rice, plus 0.25 gm. rice polish- ings }	{ 16 23 22
2	{ 4 5 6	{ 15 grm. of polished rice, plus 1 grm. of maize }	{ 23 24 35	10	{ 28 29 30	{ 16 grm. of polished rice, plus 0.5 grm. rice polishings }	{ 24 45 65
3	{ 7 8 9	{ 13 grm. of polished rice, plus 2 grm. of maize }	{ 28 34 37	11	{ 31 32 33	{ 15 grm. of polished rice, plus 0.75 gm. rice polish- ings }	{ 39 55 >120
4	{ 10 11 12	{ 11 grm. of polished rice, plus 3 grm. of maize }	{ 32 40 65	12	{ 34 35 36	{ 14 grm. of polished rice, plus 1 grm. rice polishings }	{ All over 120
5	{ 13 14 15	{ 12 grm. of polished rice, plus 4 grm. of maize }	{ 40 55 83	13	{ 37 38 39	{ 15 grm. of samp* alone }	{ 9 11 13
6	{ 16 17 18	{ 12 grm. of polished rice, plus 5 grm. of maize }	{ 55 80 >200	14	{ 40 41 42	{ 12 grm. of samp, plus 3 grm. of maize }	{ 16 19 28
7	{ 19 20 21	{ 10 grm. of polished rice, plus 6 grm. of maize }	{ All over 180	15	{ 43 44 45	{ 10 grm. of samp, plus 6 grm. of maize }	{ 36 38 75
8	{ 22 23 24	{ 8 grm. of polished rice, plus 7 grm. of maize }	{ All over 180	16	{ 46 47 48	{ 8 grm. of samp, plus 8 grm. of maize }	{ All over 120

* Samp is the rasped endosperm of maize; similar to the pearl hominy of America.

The majority of the pigeons showed typical acute polyneuritis, especially when the survival period was short. Those surviving over fifty days usually showed weakness for some time before death, and then either died in that condition (sometimes overnight) or suddenly developed an acute attack. The general results show what at first sight appears to be a clear line of demarcation just at the minimum protective quantity of supplement. Thus, with polished rice, 6 grm. or

more of maize was quite sufficient to protect indefinitely, and 5 grm. seemed just on the border-line of efficiency; two out of the three birds succumbing in less than three months, while the remaining bird was perfectly healthy and in excellent condition at the end of six months. Below this all the birds developed deficiency disease, and looking at the data superficially one is inclined to put down the range of twenty-six days for the longest survival on rice alone, and eight-three days for the longest survival on rice plus 4 grm. of maize, to individual idiosyncrasy of the birds; and to consider that the amount of vitamine in a diet makes no practical difference to the survival as soon as it falls below the minimum adequate quantity. At any rate the difference between 3 grm. of maize supplement in the case of bird 10, and no maize at all in the case of bird 3, is quite negligible, whereas the difference of only 1 grm. at the 5-grm. maize limit makes all the difference between healthy survival and collapse under deficiency disease in about two months.

It may be mentioned that we consider it desirable to extend observations over 120 days in order to be sure of survival. According to our experience, Cooper's period of fifty days is rather short, whereas if a bird survives for four months on any diet, without serious fall in weight or marked signs of weakness, it is not likely to succumb no matter how much longer feeding be continued. This period can be regarded for practical purposes as representing "infinite" survival.

The other results point in the same direction. There is, on the whole, a general tendency for the period of survival to increase with increase in the quantity of vitamine-rich supplement added to the deficient basal diet, but it is quite evident that the change is much sharper as the minimum protective quantity is reached. Thus in the rice-polishings series it happens that with .25 grm. of polishings the survival is no longer than with rice alone. On adding another .25 grm. quantity the survival is raised with bird 30, but not with bird 28. A third .25 grm. added in cage 11 make no difference to two of the birds, while for the third it makes all the difference between complete survival and collapse within two months. A fourth addition in cage 12, bringing the total supplement up to 1 grm., results in dietetic adequacy and indefinite survival.

A fairly clear difference is also brought out between samp and polished rice as basal deficient diets. Cage 7 is identical with cage 15, except that samp replaces rice, and yet all three birds survive in one case and all three succumb in the other. Elsewhere the differences are masked by the fact that the vitamine content of the diet is either definitely too high or definitely too low. But if we take cage 15 as equivalent to cage 4 we have the difference between samp and rice indicated as about 3 grm. of maize in a total ration of 15 grm., or about half that amount which when added to rice is sufficient to protect indefinitely. The suggestion is that polished rice (best table quality .22 per cent. P_2O_5) contains about half the total vitamine requirements of the pigeon—a fact which we had not at first anticipated.⁷ The relationship between samp (high-grade) and polished rice will be dealt with further, but there is little doubt that the residual vitamine in the former is considerably lower than in the latter.

⁷ Compare "The Deficiency Aspect of Maize Products." H. H. Green. This volume Fifth and Sixth Reports of the Director of Veterinary Research.

VITAMINE REQUIREMENTS IN RELATION TO QUANTITY OF FOOD EATEN.

The following Table II brings out, very clearly we think, the fact that the vitamine requirements of a bird are not absolute, but depend mainly on the quantity of food eaten, i.e. that vitamine requirements should be regarded not so much from the point of view of a "daily physiological minimum for the bird" as from the point of view of a "minimum quantity per unit of food metabolized." For purposes of comparison the relative actual quantities of vitamine in the diet are indicated in the fourth and fifth columns. For vitamine added in the maize supplement, fourth column, 1 grm. of maize represents one unit. The fifth column, denoting the probable total vitamine in the diet, is assessed on the assumption that the residual vitamine in samp corresponds to the residual phosphoric oxide, and therefore to one-fifth of the original vitamine of whole maize (*l.c.* 7). The conclusions are the same, however, whether the vitamine supplied by the supplement, or the calculated total vitamine in the diet, be considered. The experiment was carried over ten weeks, by which time the results were considered so clear that further dieting of surviving birds was abandoned.

TABLE NO. II.

Cage.	Bird.	Diet per Bird.		Survival in Days.	Relative Quantity of Vitamine in Diet.	
		Maize.	Samp.		Supplement.	Total.
17	{ 49 50 51 }	5.0	nil	{ All healthy }	5.0	5
18	{ 52 53 54 }	nil	5.0	{ 25 28 21 }	—	1
19	{ 55 56 57 }	5.0	5.0	{ 52 healthy " }	5.0	6
20	{ 58 59 60 }	5.0	10.0	{ 19 30 49 }	5.0	7
21	{ 61 62 63 }	5.0	15.0	{ 22 15 33 }	5.0	8
22	{ 64 65 66 }	5.0	20.0	{ 14 39 31 }	5.0	9
23	{ 67 68 69 }	5.0	25.0	{ 19 17 16 }	5.0	10
24	{ 70 71 72 }	7.5	22.5	{ 15 30 48 }	7.5	12

TABLE NO. II.—(continued).

Cage.	Bird.	Diet per Bird.		Survival in Days.	Relative Quantity of Vitamine in Diet.	
		Maize.	Samp.		Supplement.	Total.
25	{ 73 74 75 }	10·0	nil	{ All healthy }	10·0	10
26	{ 76 77 78 }	nil	10·0	{ 20 16 12 }	—	2
27	{ 79 80 81 }	10·0	10·0	{ 41 healthy " }	10·0	12
28	{ 82 83 84 }	10·0	20·0	{ 28 41 44 }	10·0	14
29	{ 85 86 87 }	15·0	nil	{ All healthy }	15·0	15
30	{ 88 89 90 }	nil	15·0	{ 8 16 10 }	—	3
31	{ 91 92 93 }	15·0	15·0	{ 37 61 healthy }	15·0	18
32	{ 94 95 96 }	30·0	nil	{ All healthy }	30·0	30

These results appear to leave little room to doubt that the survival of a bird is *not* conditioned by the *absolute* daily intake of vitamine, but by the percentage *proportion* of the vitamine in the food. So long as maize alone was fed, whether in 5 grm., 10 grm., or 15 grm. quantity, the birds remained perfectly healthy. The birds on 5 grm. each lost weight slowly as a result of the low diet, but otherwise showed no symptoms of malnutrition. The birds on 10 grm. and on 15 grm. fluctuated around the original weights, thus indicating that the 10 grm. quantity was quite sufficient for ordinary maintenance. Those on 30 grm. of maize per bird tolerated the high intake perfectly well and increased in weight. It may be mentioned that in those cages where the diet was raised to 30 grm. the birds were stuffed with half quantities morning and evening for the sake of avoiding undue distension of the crop, instead of being stuffed at one feeding as in the case of smaller amounts.

On the addition of samp to the diet, in amount equal to the maize moiety, deficiency symptoms may or may not be shown, but the behaviour is not conditioned by the absolute amount of vitamine in the diet. This mixture appears to be on the border-line of efficiency, and collapse or healthy survival turns on variations in the demands made by individual birds. Thus in cage 19 with vitamine intake 6, and cage 27 with vitamine intake 12, one bird succumbs and two

remain healthy, while in cage 31 with vitamine intake 18, or three times as high as cage 19, two succumb and one survives. As the proportion of samp to maize is increased the diets become definitely deficient irrespective of the absolute quantity of vitamine present, i.e. irrespective of the absolute amount of maize fed. Raising the intake of vitamine by raising the amount of maize does not enable the birds to survive, so long as the intake of deficient samp is raised concomitantly. Cage 28 gets twice as much maize as cage 20, but all six birds succumb to polyneuritis, although the birds of cage 1, with the same amount of maize as cage 20, and only half as much as cage 28, remain perfectly healthy.

Provisional Formula.—In considering the results of Tables I and II it seems to us that the data can be best harmonized by assuming—

- (a) That the function of vitamine is concerned chiefly with the metabolism of food after digestion, and only secondarily concerns the structural requirements of the bird; that although certain small absolute demands are made by the living cells as such, the bulk of the vitamine is used up in exogenous metabolism, perhaps as a catalyst in oxidative catabolism; that therefore a fixed quantity of digested food requires a fixed minimum quantity of vitamine for its combustion. This, of course, need only apply to the anti-neuritic vitamine or "water-soluble B," McCollum's "fat-soluble A," which is so important for *growth*, may be mainly a structural unit, and its requirements in animal nutrition may possibly be absolute and more or less independent of the quantity of food metabolized.
- (b) That the demand for vitamine, which on a deficient diet can not fully be met from the food itself, is met from the body reserves of the pigeon, and that as soon as the tissues are depleted below a certain physiological limit clinical symptoms of deficiency are manifested.

These assumptions concerning exogenous metabolism might be roughly expressed:—

$$S = \frac{C}{V-x} \cdot \frac{1}{K}.$$

Where S is the survival time, C the amount of vitamine which the tissues can part with before deficiency symptoms are manifested, V the amount of vitamine which is required to metabolize the food eaten, x the actual amount of vitamine present in the deficient diet, and K depends upon the quantity and quality of the food metabolized—probably the calorific value of the digested portion of the intake.

This expression omits many factors, such, for instance, as the possibility that as depletion of the body reserves approaches the danger zone there may be a tendency to conserve vitamine—to cut down waste and make a smaller amount serve the metabolism of the same amount of food; omits also factors such as washing out of vitamine reserves in elimination of water (*l.c.* 6). But for the time being it is unnecessary to complicate the expression by introducing variables which have not been investigated in detail, and we may first consider the results on the basis of the expression as it stands. Quite obviously it is not rigorous. In Table II, for instance, the survival period would work out twice as long for cage 20 as for cage 28, since the quantity of food

is only half as great, and the ratio of maize to samp the same. As a matter of fact, however, the survival periods are very similar, and the similarity requires elucidation. But the formula may still embody the cardinal facts of vitamine metabolism.

In the expression

$$S = \frac{C}{V-x} \cdot \frac{1}{K} \cdot \text{unknown factors,}$$

we cannot deal in absolute units, since there is no chemical method available for determining the amount of vitamine in any diet. We can, however, use relative numbers, based upon pigeon analysis, which will be valid throughout any given discussion. We can, for instance, select an arbitrary unit and state $V = 100$ as the average physiological requirements of the pigeon on a fixed cereal diet, i.e. the full percentage amount of vitamine which must be present in a rice or maize mixture in order that the average pigeon may remain healthy upon it; x then becomes the percentage of average physiological requirements which is actually present in the diet. When $V = x$, the diet is adequate and the survival is indefinite, no matter what values C and K may have. To determine C , the average tissue vitamine reserves which can be parted with before death ensues, in terms of V , we have to determine S , when x has some known value and K is fixed. If in cage 13, Table I, we assume that samp is vitamine-free we have $x = 0$, S averaging 11 days, and hence $C = 1100$. Calculating from cage 30, Table II, we get a similar value. Samp, however, probably still contains some vitamine, and a feeding test was therefore carried out on samp autoclaved for four hours at 135° to 140° C., this treatment being calculated to destroy any residual vitamine present. Six birds, as similar as possible in weight and appearance, were picked out and fed separately on one-twentieth of their bodyweight of autoclaved samp—weighed dry, made into a dough with hot water, and then autoclaved in covered tins. Acute polyneuritis was produced in eight, nine, ten, eleven, fifteen, and fifteen days respectively. All birds, however, developed symptoms on a full crop, and the two birds going down in fifteen days regorgitated 50 per cent. to 70 per cent. of their food from the seventh day onwards. The true intervals should probably run between seven and ten days, and we may take eight days as an average value when $x = 0$, and hence 800 as a probable average value for C . It should be noted that the behaviour of birds on autoclaved samp and unautoclaved samp is very similar, and that this figure 800 is more or less arbitrary. It might as well be taken as 1100, but, as will be seen presently, small variations in the value of C are of little consequence, and since the figure 800 fits our general data it is adhered to throughout this paper.

This number 800 is of course purely relative to the arbitrarily selected mode of expressing K . In our experiments it is valid when the diet consists of easily digestible cereal grain, such as rice or maize, in amount equivalent to one-twentieth of the bodyweight (unity for K).

INFLUENCE OF VITAMINE CONTENT ON SURVIVAL TIME.

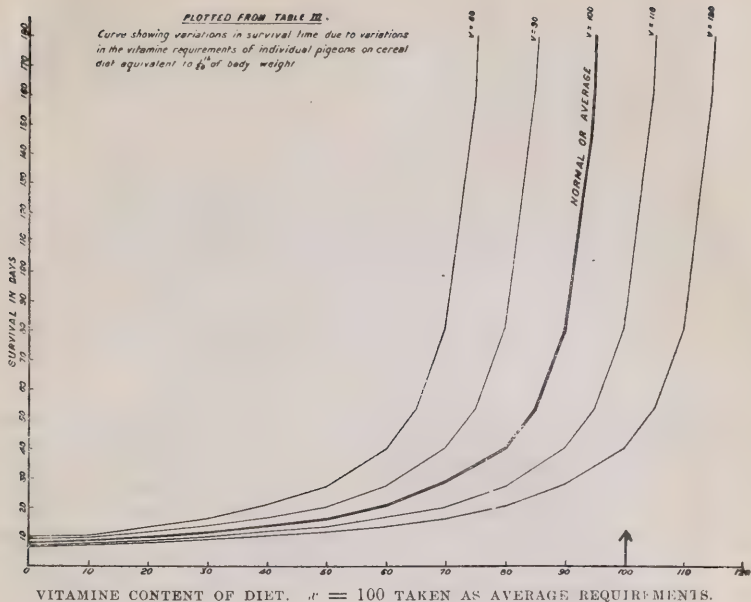
If now we disregard K for the moment and consider the effect of varying the proportion of vitamine in diets fed in the commonly used

quantity of 15 grm., or one-twentieth of the bodyweight, we can calculate the theoretical survival periods, and plot a curve showing the relationship between percentage of vitamine in the food and duration of healthy survival. Since the denominator of the expression $\frac{C}{V-x}$ diminishes with the increase in the value of x the curve is asymptotic, approaching ∞ at one end, and defined at the other end by the fixed value for C . The following numbers (Table III) show the theoretical survival periods as the amount of vitamine in the diet varies from nil to adequate, 100 being stated as the full proportion required by the average pigeon. The column $V=100$ represents the survival of the average pigeon, and in the corresponding chart is represented by the heavily lined normal curve. The remaining columns and curves illustrate the variations in survival period due to variations in V , the vitamine requirements of the pigeon. The range is only taken from $V=80$ to $V=120$, since within our experience it is rare to find two birds whose requirements vary by more than 40 per cent. from one another. C is assumed to be constant, and other causes of variation are for the moment ignored.

TABLE III

Showing variations in survival time due to variations in V , the vitamine requirements of the pigeon on a cereal grain diet equivalent to one-twentieth of its bodyweight. $x = 100$ is taken as the full percentage of vitamine necessary in the diet for health maintenance of the average pigeon; hence $V = 100$ represents the vitamine requirements of the average pigeon.

Vitamine Content of the Diet, x .	S. Survival in Days as V Varies.				
	$V = 80$.	$V = 90$.	$V = 100$.	$V = 110$.	$V = 120$.
0	10.0	8.9	8.0	7.3	6.7
10	11.4	10.0	8.9	8.0	7.3
15	12.3	10.6	9.4	8.4	7.6
20	13.3	11.4	10.0	8.9	8.0
25	14.5	12.3	10.6	9.4	8.4
30	16.0	13.3	11.4	10.0	8.9
35	18	14.5	12.3	10.6	9.4
40	20	16.0	13.3	11.4	10.0
45	23	18	14.5	12.3	10.6
50	27	20	16.0	13.3	11.4
55	32	23	18	14.5	12.3
60	40	27	20	16.0	13.3
65	53	32	23	18	14.5
70	80	40	27	20	16.0
75	160	53	32	23	18
80	∞	80	40	27	20
85	—	160	53	32	23
90	—	∞	80	40	27
95	—	—	160	53	32
100	—	—	∞	80	40
105	—	—	—	160	53
110	—	—	—	∞	80
115	—	—	—	—	160
120	—	—	—	—	∞



For the moment we need only look at the average curve for $V = 100$. It is at once obvious that small differences in the proportion of vitamin in a diet make very little difference to the survival period, when the total amount of vitamin is small. As, however, the proportion of vitamin approaches the limit of adequacy small differences make enormous differences to the survival. This fits in with the results of Table I already discussed—an extra gramme of maize makes practically no difference when only two or three grammes of maize are given as supplement to polished rice, although when the supplement approaches the adequate 5 gm. quantity an extra gramme may make all the difference between comparatively brief onset of deficiency symptoms and survival indefinitely long. From the curve it is seen that when the diet contains as much as 80 per cent. of the requisite minimum proportion of vitamin, deficiency disease still shows in less than six weeks. At this point the addition of another 10 per cent. raises the survival to nearly three months, while the addition of another 5 per cent. raises it to about six months (theoretically). If, however, the diet is so low that it only contains one-tenth of the requisite proportion of vitamin, the addition of another tenth or two-tenths makes practically no difference to the survival period—only a day or two. Even when the diet contains half the requisite proportion of vitamin an addition of 20 per cent. of theoretical requirements only makes a difference of a week or two. Thus at 40 per cent. of theoretical requirements the survival is thirteen days, and at 60 per cent. is twenty days. Differences such as these have very little significance in practical testing, since, for various reasons, individual birds can vary much more than this in their clinical behaviour. On highly deficient diets

no distinction might actually be indicated between a vitamine content of 20 per cent. and a vitamine content of 60 per cent. of average requirements. To get accurate results in practical testing of foodstuffs for their vitamine content *it is necessary to work with dieting mixtures on the border-line of efficiency.*

CAUSES OF VARIATION IN EXPERIMENTAL RESULTS.

Both from Table I and Table II it is obvious that different pigeons fed upon the *same quantity of the same diet* show very considerable variation in the period elapsing before deficiency symptoms are clinically detectable. Several explanations are open:—

- (1) The minimum physiological requirements of vitamine demanded by different individuals for the metabolism of the same quantity of food may vary. This is variation of V in the formula.
- (2) The body reserves of vitamine—C—which can be drawn upon to meet deficiency in the diet, may vary. Reserve C, of course, does not mean the total amount of vitamine in the pigeon body, but the difference between the amount present during health and the amount present at death, or at the time of an attack, i.e. means the extent of depletion which the bird can stand. The gross reserve store may vary, or birds may vary in the extent to which they can stand depletion, just as in simple starvation death may ensue with one individual sooner than with another in spite of the fact that both start off with the same amount of reserve material for general metabolism.
- (3) The time taken for symptoms to show in unmistakable clinical fashion may vary, i.e. the time taken for the clinically recognizable neuro-muscular symptoms to develop, or, one might almost say, the time taken for the tissues to degenerate histologically after the lowest physiological working level of vitamine has been reached. The deficiency may manifest itself in acute form in one case and in chronic form in another, or the disease may take a chronic course and then suddenly develop in acute form.
- (4) The influence of other factors not allowed for in the expression may vary. Thus it may easily happen that when deficient feeding begins there is wastage of vitamine, but that as vitamine depletion advances there is greater economy in utilization of remaining reserves, i.e. that the rate at which reserve vitamine is called upon to meet the demands of the exogenous metabolism may not be a uniform one, as assumed in the expression, but a diminishing one. This would cause variation in the survival period, but not necessarily to the same extent with each individual. It may also happen that there is a certain level of endogenous vitamine metabolism below which it is impossible for the bird to go; that quite independently of the exogenous metabolism the vitamine reserves steadily fall off as time goes on, and that although we may increase the general rate of depletion by feeding deficient food we cannot

decrease the normal endogenous rate by any means in our power. The expression assumes that all the reserve vitamin is used up in exogenous metabolism, and does not allow for "wear and tear" catabolism, which in general should tend to decrease the calculated survival periods. This endogenous level may vary from bird to bird just as in protein metabolism the endogenous catabolism—and excretion of nitrogen on a protein-free diet—may vary from individual to individual. Normal variation may be still further complicated by washing-out effects associated with water elimination.

The variations due to variation in the intake of deficient food will be dealt with separately in dealing with K of the formula.

A priori all these factors are bound to enter, and although it is impossible to assess the importance of each it is fairly easy to put them in order of relative importance. The first mentioned is much the most important, and, especially as the period of survival lengthens, small variations in this direction (V of formula) outweigh everything else. No matter how big the reserves may be, and no matter how economically they are expended, they are sooner or later exhausted by continued deficient feeding. No matter what fight the tissues put up, the bird cannot stand out indefinitely against consistent dietetic deficiency.

But from the practical experimental point of view the last two mentioned causes of variation may be just as important in upsetting expectations in regard to survival period on any given diet. As an illustration we may quote the curious behaviour, noted by Funk, that an acute attack, with all the characteristic neuro-muscular symptoms, may often be induced quite suddenly by mere mechanical disturbance if the bird is already on the verge of polyneuritis. Funk showed this by taking quiescent birds displaying ambiguous symptoms, swinging them round a few times in the air, and noting the sudden appearance of the clinically acute form. We have ourselves observed that the majority of early acute cases show up almost immediately after mechanical handling incident to forced feeding. Frequently, however, mere handling does not produce this effect, and a bird may show signs of weakness, regorgitation, or failure to empty the crop (necessitating cessation of feeding) long before it actually succumbs to an acute attack. Regorgitation and failure to empty the crop introduce complications due to variation in the amount of food metabolized per day, but this can to some extent be allowed for. Thus in the feeding of autoclaved samp, already mentioned, two of the birds actually survived fifteen days before developing an acute attack, although they commenced regorgitating on the seventh day. But even if allowance be made for regorgitation, one day for every 15 gm., it is difficult to know whether to put $S = 7$ or 10. Regorgitation itself is not a reliable indicator of the value of S , since some birds commence regorgitating early while others may not regorgitate at all, and one bird in a cage may teach its two fellows to regorgitate sooner than they would otherwise have done. Regorgitation or failure to empty the crop (thus preventing continuation of stuffing) *may* indicate that deficiency symptoms are about to set in, but that is all.

A more important factor in clinical variation of S is the inherent difference in form which the symptoms may take. Two birds on the

same diet may both show signs of weakness and be expected to behave in the same way, yet one may develop an acute attack and the other drag on for some weeks as a chronic case, finally dying of weakness or suddenly developing a belated acute attack. In such instances it is difficult to know whether to assign the extended survival period to lower metabolic vitamine requirements of the bird or to a higher resistance to neuro-muscular degeneration; or to a greater economy in the utilization of vitamine reserves, or to higher actual reserves in the tissues.

These causes of variation cannot very well be sifted out, and in consequence the validity of the expression is very difficult to analyse, especially on diets which are markedly deficient and on which survival is calculated to be short. As the vitamine content of the diet is increased, however, and the survival period lengthens, all other causes of variation would seem to be swamped by small variations in the value of V , the average pigeon requirements. That V itself does vary is obvious from the simple fact that one bird may succumb to polyneuritis on a diet which allows another bird to survive *indefinitely*. Thus in cage 6, Table I, one bird succumbed within two months, while another was perfectly healthy and in good condition when put out of experiment after six and a half months. This must be due to variation in individual vitamine requirements in respect to metabolism of the same quantity of food. The proportion of vitamine in the diet was inadequate for birds 16 and 17, but must have been adequate for bird 18, since the survival period was too long to allow of any other explanation. No matter how high the vitamine reserves may be they must sooner or later be depleted, and no matter how tenacious of health the bird may be it must sooner or later succumb.

If, however, we accept the diet of cage 6 as on the border-line of efficiency and put $x=100$, then the value of V may be put down as 100 for bird 18, 110 for bird 17, and 115 for bird 16; that is, the differences in behaviour can be explained by simply assuming that the requirements of the two birds which succumbed were 10 per cent. to 15 per cent. higher than the birds which remained healthy. This is the obvious assumption to make, and offers an explanation without predicated an unreasonable individual variation.

Needless to remark, differences in the extent of digestion and absorption of a foodstuff as a whole, relatively to the extent of absorption of the contained vitamine, would be equivalent to variation in x and have the same effect as variation in V .

In the formula, if we keep x constant and high, i.e. supply a diet on the border-line of efficiency, it is apparent that V is much more important than C . If the value of V for any given bird is equal to x then C can have any value at all—the diet is adequate and the body reserves need not be called upon at all. But if V varies ever so slightly above x , deficiency sets in sooner or later, no matter how high C may be. If the physiological requirements of any given pigeon only vary by 1 per cent. above the average ($V=101$) deficiency symptoms would theoretically take over two years to show, although in practice they would show in much shorter time owing to vitamine depletion by endogenous wastage. The period of two years would presuppose a uniform economical utilization of body reserves for exogenous metabolism only. Whether a bird so close to the normal as $V=101$ would show deficiency symptoms within the usual experimental

period of 120 days is not absolutely certain, but we are inclined to think that it would—probably in chronic form detectable as loss of bodyweight and generalized malnutrition. But if a bird varied by 10 per cent. above the normal ($V=110$) it would succumb in eighty days or less, and easily come within the scope of the usual period of observation.

The theoretical difference in survival between 80 and 800 days (or "infinity") need therefore only imply a difference of 9 per cent. in the individuality of the bird or a difference of 9 per cent. in the vitamine content of the diet. But if we attempted to explain such a difference in survival on the assumption of difference in C , the reserve vitamine of the tissues, we should have to assume the difference between $C=800$ ($V=100$, $x=90$, $S=80$) and $C=8000$ ($V=100$, $x=90$, $S=800$)—surely an impossible range between different birds.

If, however, x is low, the effect of small variations in V becomes relatively unimportant, and all birds should succumb in relatively short time irrespective of idiosyncrasy in individual vitamine requirements. This is brought out graphically in the chart plotted from Table III. If $x=20$, V may vary from 80 to 120 with S only varying from thirteen to eight days. But if $x=80$ the same variation of V from 80 to 120 may mean all the difference between indefinite healthy survival and collapse under polyneuritis in twenty days.

Within our experience the variation of a batch of birds in any given experiment is usually well within ± 20 per cent., and although in stray cases it may be wider, it is more frequently confined to ± 10 per cent. This is no extraordinary variation and we must always be prepared to allow for it in interpreting experimental results. Its effect on the survival period, however, may frequently be disconcerting. It shows, for example, that if we are aiming at even moderate accuracy in testing any given foodstuff for vitamine content, a fairly large number of pigeons must be utilized on a series of food mixtures (with deficient basal ration) varying uniformly above and below the normal border-line of efficiency, and that we must draw our conclusions from the birds which survive indefinitely rather than from a comparison of survival periods of limited duration. The fact that one bird succumbs in twenty-seven days on one diet and another bird survives for eighty days on another diet would at first sight suggest a marked difference in vitamine content, and we should be inclined to put the one down as highly deficient and the other as almost adequate. But as a matter of fact the two diets might almost be identical ($x=80$) and the whole difference attributed to ± 10 per cent. variation in vitamine requirements of the two birds.

Influence of C.—If we argue from the formula, and ignore all other factors, the influence of C , i.e. the tissue reserves which can be parted with before deficiency is manifested, would be directly proportional to its amount. Theoretically, if one bird possessed twice the reserves of another it should survive twice as long. Since we have no way of determining the reserves beforehand we can not say how far this expectation is realized in practice. But from the general trend of our results we are inclined to treat variations in the magnitude of vitamine reserves as of minor importance. *A priori*, since the bulk of the reserve vitamine appears to be stored in the nitrogenous tissues, muscle, liver, etc., the weight of which is more constant in proportion to the size of the bird, rather than in the

adipose tissue which is more susceptible of variation, we should not expect great variation in the quantity of reserve vitamine amongst birds previously fed on the same adequate diet. There is no reason for predicating any vitamine "depot." It does not appear likely that, of any two birds similar in weight and size, and fed upon the same vitamine-rich diet, one would store up two or three times as much vitamine as the other. And such a variation of 50 per cent. above or below the average value of C would be swamped by much smaller variations of V when x is high, and by variations in other factors when x is low. Thus if $x = 90$ a variation in V from 95 to 105 would obscure a variation in C from 400 to 1200. If $x = 30$ a variation in V from 90 to 110 would only effect a variation from thirteen days to ten days in survival, and we might therefore expect the influence of C to become more apparent. A variation in C from 400 to 1200 should then theoretically involve a variation in S from six to seventeen days. In practice, however, the resistance which different birds can put up against the appearance of clinical symptoms might obscure this. Any healthy bird at all would probably last out more than six days, and some might easily hang on for seventeen days, in spite of low reserves. Pathological symptoms take time to develop. Since variation in C is not likely to range as widely as 400-1200, real variations in experimental birds become very difficult to detect by anything short of a statistical study of a very large number of individuals.

The total vitamine content of the tissues of two birds may, of course, be the same, and yet one bird may be able to stand more extensive depletion than another before polyneuritis is manifested. Since C represents the reserves in the sense of "the amount which can be parted with before polyneuritis or death ensues," variation in tolerance of depletion can be viewed either as variation in reserves or as variation in physiological resistance.

Anomalous survival periods in experimental work, variations shown by different birds on the same diet, must frequently be explained as due to variations other than in V or C. These other factors not indicated in the formula have, however, already been discussed at sufficient length. The different causes of variation may work independently, sometimes in the same direction, and sometimes in opposite directions, and it is therefore exceedingly difficult to disentangle them.

The Significance of K in the Formula.—K was postulated as depending upon the quantity and quality of the food metabolized. If the quality of a diet be kept constant, we can investigate the effect of feeding varying quantities.

Table III leaves no room to doubt that the vitamine demands of the pigeon vary with the quantity of the food metabolized, and the preceding discussion on the importance of the percentage proportion of vitamine in any diet, constant in quantity, emphasizes the same point. The expression

$$S = \frac{C}{V-x} \cdot \frac{1}{K}$$

assumes that a fixed amount of vitamine is required for the metabolism of a fixed amount of food, and that the survival is inversely

proportional to the quantity of food, provided no other factors enter. As already shown, however, several other factors do enter, and we cannot expect a true inverse proportionality. There is the endogenous vitamine metabolism to consider, and, furthermore, it may well happen that as the quantity of food increases the utilization of vitamine becomes more economical—that the amount expended per calorie diminishes. Such factors would be fairly easily unravelled but for the fact that other causes of variation of survival time obscure experimental results. It is easy to show that increase in the gross metabolism involves increase in the demand for vitamine, but it is by no means easy to trace the exact relationship.

In 1914, in checking the observation of Funk (*l.c.* 5) we fed varying quantities of polished rice to pigeons, with results (*l.c.* 4) which may be restated as Table IV.

TABLE IV.

Cage.	A.	B.	C.	D.	E.	F.	
Diet.	Water only (Starvation).	Polished Rice Equivalent to Fraction of Bodyweight.					
		1-50th.	1-40th.	1-30th.	1-20th.	1-10th.	
Survival in days.	a	16	50	43	27	16	9
	b	14	46	29	22	16	15
	c	11	55	39	23	22	16

Unfortunately, although some of the birds regorgitated part of the food stuffed into their crops, no allowance was made, since we had in view only the general observation of lengthening survival with diminishing intake, and overlooked the possibility of a simple numerical relationship. At first glance little seems to be obtained from the figures beyond the obvious general trend. Decrease of intake is associated with increase in survival time, but the periods vary as much within each cage as they do in neighbouring cages. Thus in cage B, bird *b* varies by nine days from bird *c*, while it only varies three days from bird *a* of cage C. Similarly, although cage E got 15 grm. of rice per bird and cage D got only 10 grm.; bird *c* of cage E survived as long as birds *b* and *c* of cage D. The natural inclination was, therefore, to treat a divergence of a week to a fortnight as fortuitous, to be satisfied with the tendency towards increase of survival time with diminishing intake, very marked when we compare cages B and E, and not to emphasize any numerical relationship. If, however, we reconsider the results in the light of the assumptions now put forward it would appear that the irregularity observed was only to be expected on the diet of the particular vitamine content of polished rice, which is sufficiently low to bring down all

the birds in relatively short time, and yet sufficiently high to make small variations in V relatively important. If we take the eighteen days average survival of cage E, where $K = 1$ -20th of the body-weight or 15 grm., as basis of comparison, the survivals should run forty-five days for cage B, thirty-six days for cage C, twenty-seven days for cage D, eighteen days for cage E, and nine days for cage F. But if V varies from 90 to 110, i.e. if the birds be only assumed to vary ± 10 per cent. in their metabolic vitamine requirements per unit of food, the range of survival in days would be:—

	B.	C.	D.	E.	F.
Theoretical.....	38-60	30-48	22-36	15-24	8-12
Experimental.....	46-55	29-43	22-27	16-22	9-16

If we omit cage F from consideration, as containing the birds likely to have regorgitated most on account of the uncomfortably high intake, and at the same time likely, owing to their short survival, to be influenced too much by a few days' difference in the date of appearance of distinct clinical symptoms after tissue depletion was actually pronounced, it is apparent that the experimental data allow of the assumption that the survival is inversely proportional to the quantity of food eaten.

Fresh attempts have been made to establish this relationship, but instead of getting better results we have generally got more conflicting data, and Table IV is apparently as closely in accordance with calculated survival as we can expect to get. As a general rule extension of survival period follows diminution of intake, but the extension is not nearly so great as might be expected. Some other limiting factor, probably endogenous wastage, seems to enter in. Furthermore, the interpretation of results is beset with difficulties at whichever level of vitamine content feeding is carried out, and the birds seem to drift between the Scylla of variation in V and the Charybdis of variation in physiological resistance to clinically recognizable polyneuritis. The quantities of food which can be given are limited by the minimum metabolic requirements of the birds on the one hand, and the amount which can be stuffed without undue discomfort on the other. This gives a range of about 6 grm. to 30 grm. If we feed amounts, varying over this range, of a diet near the border-line of efficiency, normal variations in V dominate the variations in S and the variations due to K are obscured. If we feed diets very low in vitamine, the survival is short irrespective of K , and the natural resistance to the onset of clinical symptoms again seems to dominate S . If we feed diets of intermediate vitamine content, S is partly at the mercy of both causes of variation. We may, however, offer a few of our results, and interpret them as best we can.

TABLE V.

Cage.	Bird.	Diet.		Survival in Days. (Polycneuritis or Death).			Cage.	Bird.	Diet.		Survival in Days. (Polycneuritis or Death).		
		Samp. x Estimated as 30 Quantity.		Actual.	Cor- rected.	Theo- retical.			Rice, 80 ; Maize, 20. x Estimated as 76 Quantity.		Actual.	Cor- rected.	Theo- retical.
34	{ 101 102 103 }	5 gm. per bird...		{ 26 26 33 }	24 26 33	33	40	{ 119 120 121 }	5 gm. per bird...		{ 32 41 42 }	30 41 42	99
35	{ 104 105 106 }	7½ gm. per bird...		{ 17 18 25 }	17 17 23	22	41	{ 122 123 124 }	7½ gm. per bird...		{ 16 20 34 }	14 19 28	66
36	{ 107 108 109 }	10 gm. per bird...		{ 14 16 20 }	14 16 18	17	42	{ 125 126 127 }	10 gm. per bird...		{ >120 23 23 }	∞ 20 20	49
37	{ 110 111 112 }	15 gm. per bird...		{ 8 16 16 }	8 13 9	11	43	{ 128 129 130 }	15 gm. per bird...		{ 24 33 34 }	19 31 32	33
38	{ 113 114 115 }	20 gm. per bird...		{ 12 21 22 }	9 12 13	8	44	{ 131 132 133 }	20 gm. per bird...		{ 23 29 37 }	19 25 31	25
39	{ 116 117 118 }	30 gm. per bird...		{ 9 10 26 }	7 8 11	6	45	{ 134 135 136 }	30 gm. per bird...		{ 18 27 24 }	12 20 18	16

Variation
for V 110
to V 90.Rice, 80 ; Maize, 20.
x Estimated as 76
Quantity.Variation
for V 110
to V 90.Samp.
x Estimated as 30
Quantity.

Cage.

Bird.

Diet.

Survival in Days.
(Polycneuritis or Death).

Cage.

Bird.

Diet.

Survival in Days.
(Polycneuritis or Death).

Both with the samp ration and the rice-maize ration the data of Table V present many difficulties and do not demonstrate any clear numerical connection between vitamine requirements and quantity of food metabolized. The tendency towards increase in survival time as the amount of food is decreased is again quite obvious, but the individual variation of the birds obscures any exact relationship which may be there. Cage 39 is quite explicable on the assumption that a definite time, longer than the theoretical six days, is required in order that symptoms may develop. It is of interest to state that all three birds started to regurgitate on the third day of feeding, and that after the sixth day bird 118 so persistently refused to pass on food from the crop to the intestines that it could only be stuffed at intervals of two or three days thereafter. Although the attempt was made to feed 30 gm. per day (15 gm. morning and evening) the bird refused to metabolize even half of the forced intake, and although the actual survival was twenty-six days it is entered (in the corrected column of the table) as eleven days on the basis of actual food consumption. The corrected periods—seven, eight, and eleven days respectively—may merely represent the minimum time required to develop nervous lesions irrespective of the forced high intake. The same applies to cage 38, where the corrected survival varies up to five days above the theoretical. Especially with short periods it is impossible to attach much significance to a few days difference in survival. Variation in clinical behaviour can easily cover cages 37, 38, and 39, and the attempt to show differences due to variations of intake in these cages must be regarded as abortive. With the smaller quantities of food the reduced intake is clearly associated with lengthened survival, and indeed the survival might be interpreted as inversely proportional to the intake. Thus birds 101 and 102 on 5 gm. of samp survive three times as long as birds 110 and 112 on 15 gm. Similarly the corrected survivals for cage 35 are about twice as long as for cage 37. There is therefore nothing in these data to contradict the view that the vitamine demands of a bird are approximately proportional to the amount of food metabolized.

With the rice-maize series, however, the data are exceedingly irregular, and it is quite apparent that the pigeons show marked individual variation. On the 10 gm. quantity, cage 42, two of the birds succumbed to acute polyneuritis in twenty days, while the third was put out of experiment after four months in perfectly healthy condition, and with a 10 per cent. increase over its initial body-weight. There is no doubt that the diet was adequate for this bird, and that its actual vitamine requirements (V) were exceptionally low. The other two birds in the same cage must, on the other hand, be credited with abnormally high demands for vitamine or with unusually marked nervous susceptibility to tissue depletion. The 20 per cent. of maize in their ration has not helped them, and they might as well have been on polished rice alone for all the difference it makes to their survival.

The differences between cages 43, 44, and 45 might be explained on the basis of normal variation in V, and the individual variation in each cage is too great to allow any definite deduction being drawn concerning the influence of the quantity of the food fed. A variation in V of 10 per cent. above or below the normal can practically cover these cages and the influence of K is obscured. The birds in cage 43 certainly survive longer than those of cage 45, but in view of the

unexpected behaviour of cage 41 no conclusion can be drawn—although the intake in cage 41 is only half that in cage 43 the survival is actually shorter. The collapse of bird 122 after fourteen days can only be satisfactorily explained on the assumption of unusual nervous susceptibility to vitamine depletion, clinical symptoms developing very rapidly after tissue deficiency began to be felt. To attempt to attribute it solely to abnormally high demands for vitamine is out of the question, since V, if calculated from the expression on the basis $S=14$ and $K=7\frac{1}{2}$ grm., would work out at 176. This would mean that the bird would develop polyneuritis on maize alone, the diet upon which it had remained healthy for some months before the experiment began. It is possible that the vitamine demands of this particular bird were higher than usual, and that the reserve vitamine was lower than usual, but the extraordinary rapid collapse makes it certain that we are dealing with a higher physiological susceptibility to polyneuritis.

The birds of cage 40 survive longer than the birds of cage 43, but not much longer. If it were true that survival is inversely proportional to intake the former should survive three times as long as the latter. They do not, and unless we assume abnormally great differences in the individuality of the birds there is the suggestion that as the intake falls a higher relative wastage of vitamine is involved in metabolism. It may well happen that when only a small quantity of deficient food is fed the bird at first goes on utilizing vitamine for metabolic purposes at the same rate as it had been doing before the normal vitamine-rich ration was cut down, and that it does not begin to economize until its reserves have been seriously drawn upon; an initial *luxus* consumption and a later minimum consumption. We may regard the blood at any moment as in vitamine equilibrium with the tissues, excess vitamine supplied by the food being stored until the storage limit is reached, and the remainder being eliminated as superfluous. When the exogenous vitamine supply is cut off by feeding deficient food the concentration in the blood-stream would naturally fall, and the tissues discard part of their reserves in coming into fresh equilibrium, irrespective of the amount of food to be metabolized. Inevitable wastage by elimination might then occur, although as depletion of reserves goes on the tissues probably hang on to the diminishing remainder with increasing tenacity until the lowest working level of endogenous metabolism is reached.

On the view that any vitamine not used up in metabolizing food is to be regarded as so much waste we should expect the calculated survival period to be shortened as the intake is reduced. In the case of starving birds in which the intake is reduced to zero the reserve vitamine outlasts the gross reserve possibilities of the tissues, and the bird dies of simple starvation before it succumbs to specific vitamine deficiency. But if abundant water is forcibly administered (*l.c.* 6) the natural balance of tissue reserve against elimination from the blood may be disturbed, and the vitamine elimination raised to such an extent that a specific avitaminosis is manifested before death from general starvation ensues. Putting this another way, we might regard excessive elimination of water as increasing the endogenous vitamine metabolism much more than it increases the endogenous gross metabolism, or as increasing the "leakage" of vitamine irrespective of cell metabolism or endogenous metabolism proper. In feeding small quantities of food we have two things to consider—(a) the exhaustion

of vitamine reserves by withdrawal of vitamine required in the metabolism of the food, and (b) the prolongation of the period of survival so that the normal wastage fall in vitamine reserves has time to enter as a serious factor into the calculation of survival time.

In calculating survival from the formula suggested, the implication is that C is only depleted to meet the requirements of exogenous metabolism, whereas in reality C should rather be regarded as decreasing independently of K as x decreases. Provided the food is deficient, therefore, the tendency would be for S to decrease independently of K and for the survival periods to be reduced below the calculated. The reduction would be more noticeable on small intake than on large intake. For the sake of illustration we may consider the survival of two identical birds on a diet containing 90 per cent. of their theoretical vitamine requirements. If one were fed on 15 grm. and the other on 5 grm. one should survive for 80 days and the other for 240 days. But in practice this long extension of survival, as a result of reducing the intake, could not be expected, since the vitamine reserves might be depleted to the danger level much sooner by endogenous wastage. Indeed *both* birds might quite easily succumb well within the eighty days calculated for the higher intake.

In Table V the average survival is twenty-seven days for cage 43. If the birds of cage 40 on one-third of the amount of same food were identical, and variation in K the only factor to consider, they should survive three times as long, or sixty-one days. The average of the survival periods recorded is, however, only thirty-seven days. Furthermore, although the vitamine content of the diet of cage 40 is much higher than that of cage 34, the differences in survival are not very marked. This looks as if the endogenous wastage were setting a limit to the survival period irrespective of the demands made for the metabolism of the small quantity (5 grm.) of food fed.

FURTHER SERIES OF TRIALS DESIGNED TO TEST THE EXPRESSION.

As already mentioned, the expression was formulated on the basis of miscellaneous tests. A further series of feeding trials was then instituted for purposes of direct verification, the birds being picked out as uniform as possible in regard to appearance, condition, and weight. In the reserve cotes these pigeons had already been fed upon whole maize *ad lib.*, but for the sake of securing uniformity of preliminary dietary treatment they were each hand-fed on 15 grm. of ground maize (made into a dough with hot water) per day for a month before actually putting them into experiment. It was hoped that this might have the effect of minimizing differences in initial body vitamine reserves. In these tests also, special attention was paid to regorgitation from the crop, the date at which any bird commenced throwing up food being noted and a rough estimate of the amount being made. Special attention was paid to any bird which survived its cage fellows by excessive regorgitation, and the extent of rejection of food determined by collection from the bottom of the cage. In the fourth column of Table VI the survival periods therefore represent "food days." If, for instance, a bird actually died or developed unmistakeable symptoms of polyneuritis on the thirty-eighth day, but had commenced systematic regorgitation of about half its food from the twenty-fourth day onwards, the actual survival is entered up as thirty-one days. In the same way days upon which it was considered inadvisable to feed at all, owing to distension of the crop as a result of the refusal to pass on

food stuffed on preceding days, are omitted. This is admittedly not altogether satisfactory, but since regorgitation, or failure to empty the crop, is in itself not sufficient evidence of deficiency, and since the quantity of food metabolized is important, it seems to be as permissible a way as any for arriving at an estimate of the probable real value of S . Even with such correction, however, a tendency for the disease to take the chronic form may vitiate the results. It is to be regarded as a fortunate circumstance from the point of view of registering survival periods that nearly all the birds in this series developed acute polyneuritis. The results are given in Table VI.

The duration of the experiment extended over four months. It was considered superfluous to carry any test beyond this period since previous experience had shown that birds which remained healthy for four months rarely, if ever, succumb thereafter. The value of x calculated for $S=120$ days would be 94, but in practice healthy survival for this time indicates $x > 100$.

The same samples of maize meal, rice, and samp were used throughout, and accidental variation from cage to cage owing to unintentional variation of material is therefore excluded. The "autoclaved samp" is samp autoclaved at 135° to 140° C. for four hours with the intention of destroying residual traces of vitamine.

In attempting to assess the true values for x in the sixth column, i.e. the vitamine content of the diets expressed as percentage of average pigeon requirements, certain obvious difficulties are encountered. The values of x in the various cages have, of course, fixed relative significance, since each diet is made up by mixing the same materials (samp and maize or rice and maize) in varying proportions. But since the only feasible way of determining the vitamine content is by pigeon analysis, the data are subject to irregularities due to individual variation of the birds, and to get numbers which offer strict comparison of the diets it is necessary to assess definite values for the different components by considering the general run of the experiments as a whole. In column IV the probable true vitamine value of the diet is calculated by assessing the samp as 30, the polished rice as 60, and the maize meal as 160. This will be justified in discussing the table itself. Column VIII gives the experimental values of x as calculated from the survival of the pigeons concerned, on the basis of $V=100$ and $C=800$, i.e. assuming all birds to be identical. Column IX gives the vitamine requirements of each bird as calculated from the observed survivals on the assumption that all birds are identical except in regard to variation in V . The actual experimental survival periods are given in column V, the theoretical survivals to be expected on the assumption that every bird is normal being given in column VI. The range of survival to be expected on the assumption of a variation of 10 per cent. above or below average vitamine requirements is indicated in column VII, variation in V being assumed for the moment to be the only cause of variation in S .

Taking cage 5 as an example, we have 60 per cent. of samp with vitamine index 30 mixed with 40 per cent. of maize with vitamine index 160. The vitamine index of the mixture, or value for x in column IV, is therefore given as 82. In column V the actual survival periods are given as twenty-seven, fifty, and fifty days for birds 153, 154, and 155 respectively. The theoretical survival, column VI, is forty-four days, when $V=100$, $x=82$, and $C=800$, but if V varies from 110 to 90 the survival, column VII, may range from 29

to 100 days provided no other factors enter. In column VIII the experimental value of x , calculated from column V by putting $V=100$ and $C=800$, works out at seventy-one for bird 153 and eighty-four for birds 154 and 155, both of which survived fifty days. In column IX the experimental value of V , calculated from columns IV and V, keeping $C = 800$, works out at 112 for bird 153 and 98 for birds 154 and 155. In cages 15, 16, and 17, to be compared with cage 11, calculations are made on the assumption that survival is inversely proportional to intake, interfering factors being for the moment ignored.

We may now examine the data for Table VI in the light of the assumptions involved in its construction.

TABLE VI.

I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.
Cage.	Bird.	Diet.	Prob- able true value of x .	Survival in Days.			Experi- mental value of x calculated from V if $V=100$.	Experi- mental value of V , calculated from V and IV.
		1-20th Body- weight (except in cages 15, 16, and 17).		Ob- served Experi- mental.	Theo- retical for $V=100$.	Range for V 110 to V 90.		
		Samp. Maize.						
1	{ 141 142 143 }	100 0	30	{ 13 12 10 }	{ 11 }	10-13	{ 39 33 20 }	92 97 110
2	{ 144 145 146 }	90 10	43	{ 12 12 14 }	{ 14 }	12-17	{ 33 33 43 }	110 110 100
3	{ 147 148 149 }	80 20	56	{ 14 26 34 }	{ 18 }	15-25	{ 43 69 76 }	113 87 80
4	{ 150 151 152 }	70 30	69	{ 36 22 22 }	{ 26 }	19-38	{ 78 64 64 }	91 105 105
5	{ 153 154 155 }	60 40	82	{ 27 50 50 }	{ 44 }	29-100	{ 71 84 84 }	112 98 98
6	{ 156 157 158 }	50 50	95	{ 83 >120 >120 }	{ <160 }	53-∞	{ 90 >100 >100 }	105 < 95 < 95
7	{ 159 160 161 }	40 60	108	{ 78 >120 >120 }	{ ∞ }	∞	{ 90 >100 >100 }	118 <108 <108
8	{ 162 163 164 }	30 70	121	{ All over 120 }	{ ∞ }	∞	{ Over 100 }	Below 121
		Rice. Maize.						
9	{ 165 166 167 }	100 0	60	{ 19 15 24 }	{ 20 }	16-27	{ 58 47 67 }	102 113 93

TABLE VI—(continued).

I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	
Cage.	Bird.	Diet.		Prob- able true value of x .	Survival in Days.			Experi- mental value of x calculated from V if $V=100$.	Experi- mental value of V calculated from V and IV.
		1-20th Body- weight (except in cages 15, 16, and 17).			Ob- served Experi- mental.	Theo- retical for $V=100$.	Range for V 110 to V 90.		
		Rice.	Maize.						
10	{ 168 169 170 }	90	10	70	{ 19 20 35 }	27	20-40	{ 58 60 77 }	112 110 93
11	{ 171 172 173 }	80	20	80	{ 33 46 51 }	40	27-80	{ 76 83 84 }	104 97 96
12	{ 174 175 176 }	70	30	90	{ 80 >120 >120 }	80	40- ∞	{ 90 >100 >100 }	99 < 90 < 90
13	{ 177 178 179 }	60	40	100	{ All over 120 }	∞	80- ∞	{ Over 100 }	Below 100
14	{ 180 181 182 }	50	50	110	{ All over 120 }	∞	∞	{ Over 100 }	Below 110
15	{ 183 184 185 }	As in cage 11 but 1/10th bodyweight of food		80	{ 23 25 23 }	20	14-40	{ 83 84 83 }	97 96 97
16	{ 186 187 188 }	As in cage 11 but 1/30th bodyweight of food		80	{ 36 42 60 }	60	41-120	{ 67 71 80 }	113 109 100
17	{ 189 190 191 }	As in cage 11 but 1/40th bodyweight of food		80	{ 66 30 57 }	80	54-160	{ 76 47 72 }	104 (133) 108
18	{ 192 193 194 }	Autoclaved Samp. Maize. 60 40		64	{ 21 31 26 }	22	17-31	{ 62 74 69 }	102 90 96
19	{ 195 196 197 }	50	50	80	{ 40 45 72 }	40	27-80	{ 80 82 89 }	100 98 91
20	{ 198 199 200 }	40	60	96	{ 60 55 >120 }	<200	57- ∞	{ 87 85 >100 }	109 111 < 96
21	{ 201 202 203 }	30	70	112	{ All over 120 }	∞	∞	{ Over 100 }	Below 112

On the whole these results of Table VI accord reasonably well with the suppositions already advanced in proposing the tentative expression:—

$$S = \frac{C}{V-x} \cdot \frac{1}{K}.$$

Column IX shows that of the sixty-three pigeons in the experiment a numerical value for individual vitamine requirements can not be properly assigned in nineteen cases, since this is the number which survived the experimental period in good health. For two of these (cage 12) any value less than 90 will fit. For three more (cage 13) any value less than 100 (normal) will fit. In cage 8, x is 121, so that any bird of requirements below this would survive, and the range of variation between the three birds is not indicated. All three might be normal, or one might have $V = 90$, and another $V = 120$.

But of the remaining forty-four birds, twenty-one do not vary by more than 5 per cent. above or below the normal vitamine demands, seven vary from ± 10 per cent. to ± 15 per cent., while three vary even more widely. The extremes are represented by bird 149 for which $V = 80$, and bird 190 for which $V = 133$. It seems highly probable, however, that the vitamine demands for these two birds are really much closer to the normal (100) and that the anomalies in survival period are more probably due to variation in other directions. If we exclude these two we have forty-two birds out of forty-four which fit the expression within the limits $V = 87$ and $V = 112$. It is also quite likely that the nineteen birds put out of experiment in healthy condition lie within these limits and, in absence of evidence to the contrary, we could then reckon that sixty-one birds out of the total sixty-three in the experiment, fit the formula within a variation of 13 per cent. above or below normal. This may be taken as corroboration of the expression, within the limits of the experimental methods used. As a matter of fact we regard the series as a very fortunate one in the sense that the survival periods were not thrown out further than they actually are, by variations whose influence it is impossible to estimate quantitatively. Column IX, of course, really represents "*sum total of all factors stated as variation in V,*" and it does not follow that the range $V = 87$ to $V = 112$ actually indicates the true range of vitamine requirements of the birds concerned. It is possible that the range is even wider, although it is very much more probable that it is really narrower. Thus, for example, V is entered as 87 for bird 148 simply because it survived for twenty-six days instead of the theoretical eighteen days. But this difference of eight days is just as likely to be due to superior natural vigour, and for all we know to the contrary the bird may be perfectly normal with $V = 100$. In the case of bird 190 it is almost certain that we are dealing with a bird of greater susceptibility than usual to nervous degeneration, and it may well happen that its vitamine requirements are also normal; $V = 100$ instead of $V = 133$ as calculated. In some cages, of course, it is quite clear that we have to deal *only* with variation in individual vitamine requirements. Birds 157 and 158 are unmistakably subnormal, and bird 159 unmistakably supranormal, and the difference between them cannot be less than 13 per cent.— $x = 95$ is inadequate for bird 156, but

adequate for birds 157 and 158, while $x = 108$ is *not* adequate for bird 159. To determine V for any given bird, with any real approach to accuracy, would involve a succession of dieting periods in which the composition of the ration was changed every few months: beginning perhaps with $x = 110$ and dropping down to $x = 105$, $x = 100$, $x = 95$, etc.

It is noteworthy that in this series the survival periods are more closely related to quantity of food eaten than they were in the preceding table. With the exception of bird 190, cages 11, 15, 16, and 17, allow of the interpretation that the rate of depletion of body vitamine is inversely proportional to the gross metabolism. The tendency is for long survival to be reduced below the calculated, but the data are much more uniform than those offered in Table V. Thus in cage 15 the calculated survival is twenty days, and the observed survivals twenty-three, twenty-three, twenty-five days, while in cage 17 on one quarter of the amount of food the calculated survival is eighty days, but the observed survivals only thirty, fifty-seven, sixty-six days. In Table V, where the results were much less uniform, the observed survivals for cage 41 on exactly the same diet (1-40th body-weight = approximately $7\frac{1}{2}$ grm.) were only sixteen, twenty, thirty-four days. To trace accurately the influence of "quantity of food of constant vitamine content" upon the survival time, would probably involve a statistical study on a very large number of birds.

Surveying Table VI as a whole, however, we are inclined to interpret the data as substantiating the general accuracy of the assumptions involved in the formula.

Vitamine Value of the Materials Used.—In calculating the probable values of x in column IV, i.e. the proportion of vitamine in the food expressed as percentage of theoretical average pigeon requirements for exogenous metabolism of that food, indices were taken in round numbers as 30 for the samp, 60 for the rice, and 160 for the maize meal. The general run of results in Table VI indicates that these values are about as close to the truth as can be determined from the limited number of tests involved. To arrive at more accurate results, much larger numbers of birds would have to be fed upon mixtures varying more closely around the minimum adequate proportion of protective supplement to deficient base. The present data, however, are worthy of consideration.

Value for Maize.—If we accept autoclaving at 135° - 140° C. for four hours as having effectually destroyed the small residual amount of vitamine in samp we may calculate the vitamine index of maize from cages 20 and 21. If we take bird 200, which remained healthy, as having been supplied with the minimum adequate amount of vitamine, we get a value 167 for the maize component. Birds 198 and 199, however, developed deficiency symptoms, and therefore indicate maize as *below* 167. Since all three birds in cage 21 survived, maize is there indicated as having a value *greater* than 143. By assuming an intermediate round value of 160, all twelve birds—192-203—are found to vary more or less uniformly (column IX) around their own average requirements. We may, therefore, take the value 160 ± 10 as the nearest approximation we can get, and consider that the sample of maize used contains 50-70 per cent. more vitamine than is required for its own metabolism by the pigeon.

Value for Polished Rice.—Using 160 for maize as basis for calculating rice, we have in cage 12 two birds for which 30 per cent. of maize in the rice-maize ration proved adequate and one for which it did not. The maize component supplies $30/100$ of 160, or 48 per cent. of the required vitamine, and for birds 175 and 176 the remaining 52 per cent. must, therefore, have been supplied by the rice, which is thus indicated as containing $52 \times 100/70 = 74$ per cent. of minimum requirements. Even if we assume so high a value for maize as 200, rice would still be indicated as high as 57. These two values, 200 for maize and 57 for rice, would then give too high a total in cage 11 to account for the rapid collapse of bird 171. But if we keep to 160 for maize and assume that Birds 175 and 176 are subnormal to the extent of 10 per cent. in vitamine requirements, the rice is indicated as 60. From cage 13, in which all three birds survived, if $160 \times 40/100 = 64$ per cent. of the total requirements are supplied by the maize component, and the remaining 36 per cent. (required to render the diet adequate) by the rice, the latter is again indicated as 60. As reference to columns IV, VIII, and IX shows, the value 60 for polished rice fits the general results of the rice-maize cages fairly well, and evades the necessity for assuming unnaturally wide variations in the individual idiosyncrasy of the birds. It may, therefore, be taken as *highly probable* that highly polished table rice *may* still contain, as residual vitamine not removed with the sub-pericarpal layers in the process of polishing, from half to two-thirds of the amount of vitamine required for its own metabolism by the pigeon. It must be emphasized that the proof offered for this is not claimed to be rigorous, but only carries a high degree of probability. The disconcerting element in our conclusion is the fact that we have not so far succeeded in verifying the value 60 for polished rice, by autoclaving the *rice component only* of a rice-maize ration. Our experiments in this direction (autoclaving different components of an efficient mixture) have hitherto been scanty and ambiguous, but they are being continued and will be reported upon later. Meanwhile we have developed the impression that in the destruction of vitamine in any given foodstuff several factors may be involved, of which one may be the secondary changes of other compounds present. Thus it is not yet absolutely certain that a temperature which destroys vitamine in the pericarp of a grain will destroy vitamine (if any) in the starchy endosperm. The reaction of a mixture, potentially acid or alkaline, is known to affect the thermostability of contained vitamine. There is also the possibility to consider that although vitamine is destroyed as an entity by autoclaving, split products remain which can to some extent "spare" intact vitamine subsequently added.

But if we do not admit that polished rice still contains appreciable amounts of residual vitamine, our results become intensely difficult to harmonize with one another. We would then have to assume very wide and very *erratic* variations in individual vitamine requirements, and the drawing of any conclusions at all from pigeon experiments would be beset with increased difficulties.

Value for Samp.—A comparison of cages 1-8 with cages 9-14 at once suggests that samp contains considerably less vitamine than polished rice. In discussing Table I the same difference was noted, and we may add that in every series of experiments in which we have

compared samp and rice as deficient basal ration, the proportion of vitamine-rich supplement required for the former was greater than that required for the latter. The number of tests involved is now large enough, we think, to put the differences noted beyond the sphere of coincidence or chance.

In Table VI, if we equivalent cage 12 to cages 6 and 7, we note that a rice-maize mixture containing 30 per cent. of maize is as efficient as a samp-maize mixture containing 60 per cent. of maize. The actual value for samp is rather difficult to determine, however, since any assumption of a definite value for the maize component throws the whole "individuality error" of the pigeon on to the samp component; and, since the latter contains so little vitamine, its value may be disproportionately raised, or overlooked altogether. In other words, the vitamine content of samp is so low that the influence of the quota contributed to the diet by the samp component is apt to be smothered by the idiosyncrasy of the birds. Thus, if we keep to the value 160 for maize, and in view of the collapse of bird 159 assume that birds 160 and 161, its cage fellows, receive the bare minimum of vitamine, we get samp indicated as practically vitamine free. If, on the other hand, we calculate from birds 157 and 158, we get samp indicated as containing 40 per cent. of theoretical pigeon requirements. A comparison of cage 6 with cage 19 suggests 17 per cent. to 33 per cent. On the whole the treatment of samp as still containing a quarter to a third of the vitamine required for its own metabolism by the pigeon, enables the general data to be harmonized most successfully. This, naturally, only applies to the particular high grade of samp used in these experiments. Some less perfectly machined samples of samp obviously contain fragments of pericarp still adhering to the endosperm, and in such cases the vitamine index might well be higher.

It may be mentioned that the P_2O_5 content of the samp used was .1 per cent. In a companion paper (*l.c.* 7) we have expressed the belief that the location of vitamine follows the location of phosphorus in any given maize kernel, although at the same time showing that no general correlation exists between vitamine and phosphorus in different kernels. If that contention is true we have good reason for supposing that the samp used contains at least a quarter of minimum avian requirements. Vitamine index 25 for samp containing .1 per cent. P_2O_5 corresponds to vitamine index 160 for a mother grain containing .64 per cent. P_2O_5 —a figure above the average for South African grown maize.

It is interesting to note in passing that the sample of polished rice used (.22 per cent. P_2O_5) contained rather more than twice as much residual phosphorus as the sample of samp. From Table VI we have concluded that the rice contained twice as much residual vitamine as the samp. Is this coincidence?

ADDENDA.

* *Absolute Values.*—It would be exceedingly interesting if the proportion of vitamine required in metabolism or present in any diet could be expressed in absolute terms. Although it is impossible to do

this with any real approach to accuracy it is still worth while indicating maximum figures, if only for the sake of emphasizing how small absolute requirements really are. If, in a speculative attempt at evaluation, we take the vitamine isolated by Funk as the nearest approach to the pure compound so far obtained, and consider his statement that 4 to 5 mg. of his original crystallization from the vitamine fraction of the extract from rice polishings sufficed, when injected intramuscularly, to effect the complete cure of polyneuritic birds in such a fashion as to permit of their being fed upon polished rice for a further period of five to six days, we would have 1 mg. per day of his crude vitamine indicated as more than sufficient to meet the deficiencies of 15 to 20 gm. of polished rice. If we assume that the residual vitamine in the polished rice used amounted to about half that required for its own metabolism we would have the minimum requirements of the pigeon indicated as less than 2 mg. in 20 gm., or *less than .01 per cent.* in the diet. But since, of the few milligrams injected for curative purposes, some may have been wasted by elimination and only part stored for subsequent use in metabolism, and since the product used was still far from pure (still separable into at least two components), this estimate is much too high. Seidell has estimated that a diet should contain rather less than .003 per cent. of vitamine, but even this minimum may still be too high.

If we attempted to assign an absolute value for V ($V=100$) in our formula we should therefore have to put it down as less than .003. Whole maize, $x=160$, would then be credited with containing about .005 per cent. of vitamine.

If the demand for vitamine is really proportional to the gross energy metabolism the absolute demands of the pigeon would work out at less than one-hundredth of a milligram per Calorie.

Extent of Body Reserves.—If we accept the number 800 for C when the pigeon is fed upon a cereal diet amounting to one-twentieth of its own body-weight, then the body reserves are only sufficient to meet the deficiencies of eight-twentieths (or 40 per cent.) of the body-weight of vitamine-free food. The reserves may therefore be equvalated to the amount of vitamine contained in about a quarter of the pigeon body-weight of maize. Thus, if a bird weighing 300 gm. be fed upon 15 gm. of vitamine-free cereal (autoclaved samp, say) it should theoretically exhaust its reserves in eight days. By this time it has metabolized 120 gm. But if this 120 gm. consisted of 75 gm. of whole maize and 45 gm. of autoclaved samp the diet would be adequate and the reserves need not be drawn upon at all. The reserves may therefore be equvalated to 75 gm. of whole maize, or a quarter of the body-weight. Expressed in terms of fresh butcher meat the equivalent would be about 300 gm., i.e. the body-weight. Expressed in absolute units the reserves would amount to less than 4 mg. of vitamine.

“Reserves” of course mean the amount which can be parted with before deficiency is manifested, and not the total amount in the tissues of the bird. The flesh of birds dying of polyneuritis is far from vitamine-free, and it is indeed probable that polyneuritic flesh would still be dietetically adequate for healthy pigeons (*l.c.* 4, p. 25). It seems probable that death would ensue as soon as the “vitamine index” of the tissues of the bird fell below 100, i.e. as soon as the vitamine fell below that required for the normal catabolism of the bird’s own flesh.

In regard to the locus of the reserves nothing very definite can be said. It has been shown that liver tissue is richer in vitamine than muscle, but it does not follow from this that the liver acts as a special vitamine depot. It is more probable that there is no special depot, but that under normal conditions the reserves are scattered over all the tissues, the muscles owing to their mass accounting for the greater part.

Vitamine Index.—We have coined the term "vitamine index" as indicating not the absolute proportion of vitamine in a foodstuff, but the proportion relatively to the gross energy value of the digested portion of the food. Thus butcher meat and maize lie fairly close in respect to vitamine index and both contain well over 50 per cent. more vitamine than is required for their own metabolism by the pigeon. But owing to the amount of water present in meat its actual vitamine content is much lower than that of maize. In the same way diets containing much cellulose (straw or hay) may be low in vitamine content, but still have a high vitamine index when the proportion of vitamine absorbed is considered in relation to the small amount of available digestible matter. Thus in considering dietaries of farm stock one should be very chary of postulating vitamine deficiency in natural vegetation of low general nutritive value.

Furthermore, the absolute amount of vitamine which a bird receives is dependent upon the quantity as well as upon the nature of its diet, whereas the vitamine index depends only upon the nature, and, as in cages 15 and 17 of Table VI, the absolute intake may be quadrupled while the vitamine index remains the same. According to our view it is mainly upon the vitamine index that the health of a bird depends. If it can be shown that the daily vitamine requirements are absolute and not, as we maintain, relative to the gross energy metabolism, then the term "vitamine index" would cease to have any particular meaning.

It should be noted that in using the term "gross energy metabolism" we are on uncertain ground. It may be that the proportion of vitamine required for the metabolism of carbohydrate is not the same as that required for the metabolism of its dynamic equivalent of protein or fat, but since protein and fat definitely do require vitamine for their metabolism the hypothesis of isodynamic equivalence may be taken as working basis until such time as positive evidence is forthcoming. The question hardly comes into the tests recorded here, since our diets were all cereal and similar in character.

The Nature of Polyneuritis.—In the earlier stages of work on beri-beri and polyneuritis much discussion raged as to whether the symptoms were traceable to "deficiency" or to "intoxication." It may be, however, that in the last resort this distinction disappears and that deficiency merges into intoxication. It is now agreed on all sides that the primary cause is a deficiency in the diet and not the presence of toxic material. Nevertheless the possibility should not be lost sight of that toxic intermediate metabolites may be formed under conditions of imperfect exogenous catabolism in absence of adequate vitamine, and that these may play a part in producing the syndrome of polyneuritis. It seems more rational to regard the progressive nervous degeneration as caused by gradual depletion of structurally necessary vitamine, but intoxication may also be held responsible. In considering the acidosis arising on an exclusive diet of fat we do not

always think of it as a "deficiency disease" (deficiency of carbohydrate), but often think of it as an "intoxication," and in such an acidosis intermediate products of fat catabolism can readily be detected in the urine. It might possibly be that in polyneuritis the nerves are not actually suffering from vitamin depletion at the time of an attack, although naturally the depletion hypothesis is the simpler.

Vitamine Requirements for Various Classes of Animals.—Very little work appears to have been carried out specifically in this direction, and it is not yet possible to say much concerning the vitamin requirements (V of the formula) of different classes of animals. At the same time an unjustifiable tendency is frequently shown in the literature to assume that the requirements of all animals are the same and to ignore the possibility of specific quantitative differences.

In the case of *cattle* we have little doubt that the exogenous vitamin demands are much lower than they are in the case of pigeons. Earlier data (*l.c.* 4) from this laboratory have been adduced to show that deficiency disease can not be produced in cattle even after feeding on an almost exclusive diet of polished rice for over a year. If pigeon data could be applied to cattle, deficiency disease should have been pronounced in three or four months. In a few feeding tests on cattle which have since been carried out, and which will be reported upon later, samp was used as basal ration, and from these experiments we hope to be able to demonstrate that the requirements of cattle in antineuritic vitamin are not greater than half those of pigeons.

So far as the data of experiments on *rats* upon "*synthetic diets*" allow of quantitative interpretation it would appear that the maintenance demands for antineuritic vitamin are also considerably lower than in the case of pigeons.

In regard to *human* requirements it is customary in the medical literature to treat man and bird as equivalent, and to assume that if any given diet is adequate or inadequate in respect to antineuritic vitamin for the pigeon it is adequate or inadequate for man. This is probably true, and although it is very difficult to demonstrate the parallelism accurately it is fairly certain that human and avian demands do not vary widely from one another. One might perhaps put it that the normal variations in V of pigeon and man overlap one another. It happens that in the literature dealing with beri-beri the supplementary components of "*border-line diets*" have not been tested upon pigeons with sufficient accuracy to render close comparison possible. Thus the diet upon which beri-beri broke out in Frazer and Stanton's^{*} experiment at Durien Tipus consisted of polished rice 21.3 oz., dried salt fish 4.25 oz., onions 1.75 oz., potatoes 1.75 oz., coconut oil 0.85 oz., coconut 1.5 oz., tea 0.12 oz., salt 0.1 oz. If we omit the tea and coconut oil as containing only negligible amounts of vitamin, and calculate the dry matter in the remaining supplement on the basis of the moisture content given in the text, we get a dry-weight supplement of nearly 4 oz. The vitamin content of this must be fairly high, but if we express minimum avian requirements as 100 it can not very well be above 300 nor below 200. The value 220 would confer upon the whole ration a vitamin content of about 80 per cent. of avian requirements, while to bring the whole ration to the border-line of efficiency

^{*}Frazer and Stanton: "The Etiology of Beri-beri. Studies from the Institute of Medical Research, Federated Malay States, No. 12, 1911."

we would have to assume a value as high as 360—a figure certainly too high. A value in the neighbourhood of 200 represents the nearest estimate (or guess) which one can make. With the corresponding vitamin index 80 for the whole ration the survival of pigeons, calculated from the formula, works out at forty days (twenty-seven to eighty days) when the amount of food fed is equivalent to one-twentieth of the body-weight. If we take the whole ration in Frazer and Stanton's study as equivalent to 27 oz. of rice, and the average weight of the labourers as 100 lb. (p. 13) the proportion fed to the men works out at about one-sixtieth of the body-weight. On strict pigeon analogy the average period before onset of beri-beri should be four months, and the range of survival eighty-one days to eight months if individuals vary by ± 10 per cent. in their vitamin requirements. The first case is actually recorded after eighty-seven days and a few more cases occur shortly after. The diet was of course changed after the outbreak so that only the men varying in the direction of high requirements actually showed beri-beri. From Frazer and Stanton's data, therefore, it is legitimate to assume a close parallelism between human and avian requirements; at any rate the data *do not allow us to detect any difference*.

The limitations of available records are such that although they allow us to *assume* that the expression suggested for pigeons is applicable to man, they give no clear assurance of strict parallelism. The analogy in survival time may only be apparent, and it may well be that other variables (physiological resistance, etc.) which make pigeon records so difficult to interpret render comparison with human records unreliable. Such comparison on the basis of survival time is only offered in speculative fashion and is, of course, recognized as open to fallacy.

Aron⁹ quotes the case of the steamship "Knight Templar" sailing from Bombay, the crew of which were prohibited by their religious laws from eating meat except when killed in a special way, and in consequence were confined almost exclusively to a rice diet. On revictualling at Liverpool only highly polished white rice was obtainable. This was given to the crew on 25th July, and by 25th September cases of beri-beri began to occur, while by 25th October eleven men were down. Thereafter unpolished rice was procured and the outbreak ceased. The total crew is not specified, but the fact that cases occurred in two months, and rapidly increased during the third, is of interest. Assuming an average intake of rice equivalent to one-sixtieth of the body-weight the period of survival, calculated upon the pigeon basis, should be two months—seven to twelve weeks if individual variation remained within ± 10 per cent. of the normal.

A diet upon which beri-beri was rife in the Japanese army during the Russo-Japanese war is given by Takaki (quoted Frazer and Stanton l.c. 8) as 30 oz. of rice and 5 oz. of meat. Such a diet, from the pigeon point of view, is little better than rice alone and allows of no close comparison between human and avian requirements. The diet upon which the navy before Port Arthur showed not a single case of beri-beri consisted of 20 oz. of rice, 10 oz. of barley, and 16 oz. of meat. If the barley in this ration were "pearl barley," *which may itself be*

⁹ Aron: "Phosphorus Starvation with special reference to Beri-beri," *Phillipine Journal of Science*, Vol. V, No. 1, 1910.

deficient,† and the rice highly polished, the diet would be deficient for pigeons and human requirements therefore indicated as lower than avian. If, on the other hand, "whole barley" is meant, the diet is comfortably over avian requirements, and human requirements can still be regarded as the same. On the whole, the quantitative parallelism between avian and human maintenance requirements must be regarded as fairly close in respect to antineuritic vitamine or "water-soluble B" of McCollum. Whether this parallelism extends to requirements in other directions is far from certain, and indeed it seems highly probable that specific differences of phylogenetic origin exist. McCollum has shown that whole cereal grain is deficient in "fat-soluble A" for *growth* requirements for young rats. In regard to *maintenance* requirements of pigeons there would seem to be no such deficiency, since we have kept pigeons perfectly healthy and in excellent fat condition for periods of a year upon maize alone.

The data concerning minimum maintenance requirements of adult rats and minimum growth requirements of young pigeons are still very scanty, and the comparative study of the demands at different periods of life offers an interesting field of work. So far as we have been able to gather from the literature available to us up to the date of writing it would seem probable that "fat-soluble A" is dominantly a growth hormone of which more is needed in youth than in adult life. According to our present view the antineuritic vitamine is dominantly a maintenance hormone largely concerned with exogenous metabolism, and it is therefore not improbable that the proportion (percentage) which must be present in the food is much the same throughout life.

SUMMARY.

The results of an extensive series of tests upon pigeons are recorded on diets varying in known fashion in respect to antineuritic hormone or "water-soluble B," and an attempt is made to correlate the survival periods of the birds with the proportion of vitamine present in the diet.

It is shown that the daily demand for vitamine is not absolute, but that it depends very largely upon the extent of exogenous metabolism. If the absolute amount of vitamine given per day is kept constant, polyneuritis is induced when the amount of deficient basal ration is sufficiently increased. From this it is concluded that the main function of vitamine concerns the gross metabolism of food—probably oxidative catabolism. As yet there is no evidence to justify the view that vitamine consumption is related to carbohydrate metabolism more specifically than it is to metabolism of protein and fat. Vitamine is also regarded as required for structural purposes by the various cells of the tissues, but the greater proportion in the food is used up to metabolize the food itself. It is, therefore, the ratio of

† In some of our own experiments on pigeons the ordinary household barley, sold for the consumption of Europeans, produced polyneuritis in as short a time as polished rice itself. The recommendations of barley as protective supplement of special virtue, so frequently made in the medical literature, are very ambiguous. If ordinary grocer's barley is meant the supplement may be valueless and the recommendations misleading. If whole barley is meant any other whole cereal grain would serve the same purpose.

vitamine absorbed in digestion to the energy value of the digested portion of the food which is suggested as determining the efficiency of a diet. The term "vitamine index" is used to denote this ratio, the number 100 being taken as representing average vitamine requirements of the pigeon, and vitamine indices being expressed relatively. Thus, maize = 160 indicates that whole maize grain contains 60 per cent. more vitamine than is required for its own metabolism.

When the diet is deficient the vitamine required for its metabolism is taken from vitamine reserves stored up in the tissues of the pigeon and the period of onset of deficiency symptoms depends upon the rate of depletion of reserves, which in turn depends upon the vitamine index of the diet and the amount of the diet metabolized.

The validity of the simplest formula expressing these facts is tentatively discussed:—

$$S = \frac{C}{V-x} \cdot \frac{1}{K} \cdot \text{other factors.}$$

Where S is the period elapsing before onset of deficiency symptoms, V the minimum proportion of vitamine in the diet necessary for health, x the proportion of available vitamine actually present in the diet, C the extent of vitamine reserve in the tissues, and K the quantity of food metabolized. It is shown that such an expression is far from rigorous and does not hold in individual cases, but evidence is adduced to show that it can be regarded as embodying the cardinal facts of vitamine utilization in exogenous metabolism. It is particularly difficult to demonstrate the variation in survival due to variation in K, but if K is increased in such a way as to reduce x the influence on survival becomes more clearly marked. In any given case there are so many interlacing factors which can influence the duration of survival of a pigeon that it is impossible to predict the onset of polyneuritis with any assurance, but when a large number of birds is dealt with the general run of survivals can be prophesied with fair certainty. Some of the causes of variation are discussed. Endogenous wastage of vitamine tends to reduce the calculated survival period, especially when K is small, and in general experimental survivals are shorter than anticipated. A lower limit to S may be set by the fact that symptoms take time to develop. Variations in physiological resistance to clinical manifestation of deficiency make S difficult to determine experimentally. Variations in V, the minimum vitamine requirements of the birds, influence the survival periods very markedly when x is high, and hardly at all when x is low. On a deficient diet relatively little difference in survival may be effected until the vitamine content is raised almost to the border-line of efficiency.

Any attempt to deduce the relative antineuritic efficiency of two diets by comparing duration of survival of pigeons in experiments of short duration is open to a summation of errors. In determining the vitamine content of various foodstuffs accurate data can only be obtained by feeding mixtures which protect indefinitely (a few months), and as many birds as possible should be fed just above and just below the border-line level of efficiency in order that variation in V may be ruled out. In our own experiments variation in V generally fell well within ± 13 per cent. of the average value, but could frequently be interpreted as being wider.

The extent of the body reserves in pigeons, or extent of vitamine depletion which the tissues can stand before deficiency is manifested, is assessed as equivalent to the amount of vitamine contained in about one-quarter of the body-weight of whole maize. In absolute units this probably corresponds to only a few milligrams of vitamine at most.

Polished rice, the staple deficient basal diet in experimental work on beri-beri, is regarded as containing residual vitamine equivalent to at least half of that required for its own metabolism by pigeon or man. The vitamine requirements of man and pigeon are regarded as very similar, and much higher than those of cattle.

g.

